Mantra Snap Software

User's Manual for Mantra Snap 1.0

PerkinElmer

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This manual describes Mantra Snap version 1.0 software.

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1 Welcome to Mantra

The topics in this part of the User's Manual provide general information about the Mantra™ Quantitative Pathology Imaging System. It is important to read and understand the General Cautionary Statements before using the system. The Hardware Overview provides a description of each of the hardware components that constitute the Mantra system. Please review the Fluorescence Illuminator User Information so that you are familiar with the fluorescence illuminator and its safety precautions.

The Mantra system is an easy-to-use, semi-automated quantitative pathology workstation for the development of multiplexed immune cell and expression profiling assays in situ. It includes inForm® Advanced Image Analysis Software that enables immune cell phenotyping within the tumor microenvironment in FFPE tissue sections.

The Mantra Snap software provides rapid and intuitive image capture, and works with the controls on the Mantra instrument itself. The system can be configured so that the images you take are automatically and seamlessly transferred to the inForm software for segmentation and phenotyping in the background while you continue to take more images.

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1.1 Hardware Overview

The Mantra Quantitative Pathology Imaging System consists of the Mantra instrument and Mantra Snap and inForm software. You can take pictures by using the Mantra Snap software or by using the controls on the instrument while looking into the eyepieces. You can even configure the Mantra Snap software to automatically send your images to the included inForm Tissue Finder software for processing and analysis while you continue to take more pictures.

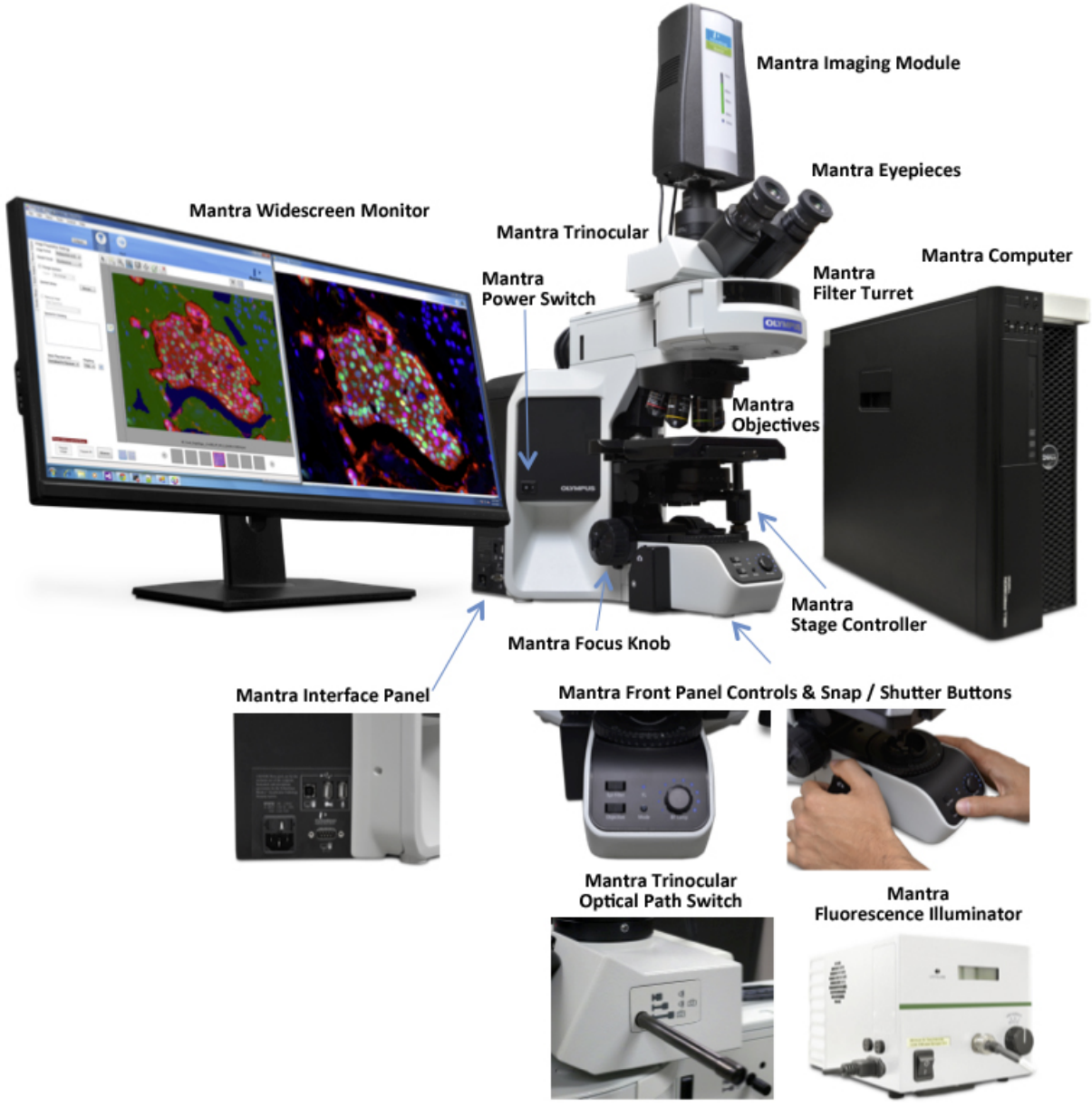

Figure 1. The Mantra Quantitative Pathology Imaging System with important components identified.

- **Mantra Imaging Module**: contains a cooled, scientific-grade camera system and PerkinElmer's solid-state tunable imaging filter, as well as circuitry for USB control of the instrument. A green linear LED display on the front panel indicates the current wavelength as well as when the system is initializing.

- **Mantra Eyepieces**: allow you to see your slide directly
- **Mantra Trinocular and Optical Path Switch**: diverts the image from your slide to either the eyepieces only, the camera only, or to both eyepieces and camera (shared)
- **Mantra Epi-Fluorescence Filter Turret**: a motorized turret containing epi-fluorescence filters

  IMPORTANT: Do not move the epi-fluorescence filters from their designated and marked positions unless directed to do so by a PerkinElmer technician.
- **Mantra Power Switch**: main power switch for the Mantra instrument
- **Mantra Objectives**: a motorized turret containing your microscope objectives

  IMPORTANT: Do not move the objectives from their designated and marked positions unless directed to do so by a PerkinElmer technician.
- **Mantra Stage Controller**: moves the microscope stage. There are separate knurled knobs to allow movement along both the X and Y axis
- **Mantra Focus Knob**: allows coarse and fine focus of your slide on the Mantra microscope stage
- **Mantra Front Control Panel**: contains switches for changing the Mantra's epi-fluorescence filters and objectives, a button to change modes from brightfield (BF) to fluorescence (FL), and a large knob surrounded by active indicators to adjust the illumination level
- **Mantra Snap/Shutter Buttons**: located on a hand controller to manually snap pictures and open or close the Fluorescence Illuminator shutter
- **Mantra Interface Panel**: located on the left side of the Mantra instrument (as you sit in front of it), contains the power input connector and fuse, USB ports for the computer, optional footswitch (not yet enabled), and optional microphone (not yet enabled), and an RS232 port for control of the microscope objective and epi-fluorescence filter turrets, as well as the shutter.
- **Mantra Computer**: comes with the Mantra instrument and has the Mantra Snap and inForm software installed and activated at the factory. Also included are a wireless keyboard and mouse
- **Mantra Widescreen Monitor**: ultra-widescreen monitor enables side-by-side viewing of both the Mantra Snap and inForm program windows

1.2 System Specifications

Mantra Instrument	
Imaging Modes	Brightfield and fluorescence, multispectral and color imaging
Microscope	Customized research-grade Olympus microscope with: • 8-position motorized epi-fluorescence filter turret • 7-position motorized objective nosepiece • Active management of intensity-per-objective in brightfield • Non-mechanical sensing of trinocular selection • Integrated ergonomic controls on microscope base
Camera type	Multispectral 12-bit camera using cooled scientific-grade CCD
Camera spectral range	• 440-720nm for brightfield imaging and visible fluorescent dyes • 770nm for near-infrared (NIR) dyes such as Cy7, Alexa Fluor 750, and similar
Objectives	4X / 0.16 NA, 10X / 0.40 NA, 20X / 0.75 NA (40X / 0.95 NA optional)
Pixel Resolution	0.25 micron (optional 40X), 0.50 micron (20X), 1.0 micron (10X), 2.5 micron (4X)
Epi-fluorescence filter cubes	DAPI, FITC, TRITC, Cy5 Cy7 (optional) Quad-band for DAPI, FITC, TRITC, Cy5 (optional for eyepiece visualization)
Fluorescence illumination	Metal hallide 200W lamp with liquid light guide, 2000-hour bulb life
Brightfield illumination	High-output, color balanced white LED

Physical Dimensions	
Mantra Workstation (W x H x D)	163 x 76 x 56 cm (including Mantra, fluorescence illuminator, monitor, computer, keyboard, mouse) 145 x 76 x 56 cm (as above without computer)
Weight	24 kg (Mantra instrument only)

Computer (minimum specifications)	
General	Quad-core, 16 GB RAM, i5 processors, mini-tower style
Hard disk drives	Two: 500 GB for operating system and programs, 1 TB for data storage
Operating system	Windows 7, 64-bit, .NET 4.5
Interfaces	Gigabit Ethernet, USB
Monitor	29" flat panel LCD, 2560 x 1080 pixels

Software	
Acquisition software	Mantra Snap (1 seat)
Image analysis software	inForm Tissue Finder (2 seats)

1.3 System Requirements

Mantra System Power Requirements

Mantra Instrument	
AC input power	100-230 VAC, 1.0A, 50/60Hz
Fuses	2x 2.0A, 250V (P/N CLS 139063)

Mantra Fluorescence Illuminator	
AC input power	100-240 VAC, 2.0A, 50/60Hz

Mantra Fluorescence Illuminator	
Fuses	2x 5.0A, 500V

Mantra System Environmental Requirements

Mantra Instrument	
Operating Environment	Indoor
Operating Temperature	18-28° C
Operating Humidity	50% maximum, non-condensing
Operating Altitude	6561 feet (2000 meters)
Storage Temperature	15-30° C
Storage Humidity	50% maximum, non-condensing
Shipping Temperature (24 hours nominal)	-10-45° C
Pollution Degree	2

1.4 Operator and Equipment Safety

It is the responsibility of the purchaser to ensure that all persons who operate the Mantra Quantitative Pathology Imaging System are aware of the following cautionary statements. As with any scientific instrument, there are important safety considerations, which are highlighted throughout this User's Manual.

General Cautionary Statements
READ AND UNDERSTAND THIS USER'S MANUAL BEFORE ATTEMPTING TO OPERATE, TROUBLESHOOT, OR MAINTAIN THE MANTRA SYSTEM. READING THIS MANUAL FIRST MAKES IT EASIER AND SAFER TO OPERATE AND MAINTAIN THE SYSTEM.

- The Mantra system contains a motorized objective changer and a motorized epi-fluorescence filter changer. Always keep your fingers, long hair, loose clothing, dangling jewelry, etc., away from the objective and filter changers to avoid personal injury and possible system damage.
- Use only as specified by the operating instructions. The protections may be impaired if the equipment is used in a manner not specified by the manufacturer.
- Do not operate the system if there has been a malfunction of any component, as this could lead to equipment damage or physical injury. Contact PerkinElmer for assistance.
- Operate the system on a flat, stable surface.
- Do not drop the Mantra imaging module, illumination module, or any other system component.
- Do not expose the imaging module to prolonged heat above 40°C (104°F).
- Do not operate the system in an environment with explosive or flammable gases.
- Do not operate the system in places where it may be splashed with liquid.
- Use only the power supply cord set provided with the Mantra system. If the correct cord set for the location was not provided, please contact PerkinElmer for a replacement. Do not use power supply cords with inadequate ratings.
- Some cables and adapters supplied with the system have proprietary specifications. Do not connect components supplied by PerkinElmer using unqualified cables or adapters. Doing so could result in damage, and voids the Warranty. Contact PerkinElmer for a replacement if necessary.

- Use only a properly grounded power outlet when connecting the system to power.
- Place equipment and devices in a manner such that the power switches and disconnecting devices are accessible at all times.
- Follow the recommended maintenance procedures to help ensure optimal performance.

Caution: Installing, servicing, and moving of the Mantra system should be performed by PerkinElmer authorized and trained personnel only. Power must be disconnected from the system before servicing.

1.5 Important Safety Information for the Fluorescence Illuminator

Before using the illuminator, please read all warnings and operating/safety instructions in this User's Manual. Keep this manual in a safe place for future reference.

- Do not use the illuminator for purposes other than its intended use. Doing so could cause damage to the unit and/or personal injury, and may void the warranty.
- Do not expose the illuminator to water or moisture.
- Do not expose the illuminator to extreme heat or cold.
- Do not expose the illuminator to open flames.
- Do not allow objects to fall on or liquids to spill on the illuminator.
- Use only the AC power supply cord set provided with the illuminator. If the correct cord set for the location was not provided, please contact PerkinElmer for a replacement. Do not use power supply cords with inadequate ratings.
- Connect the AC power cord only to the designated power sources as marked on the illuminator.
- Make sure the electrical cord is located so that it is not subject to damage.
- Always make sure the illuminator is disconnected from power before installing the bulb, connecting components, or cleaning the exterior surfaces.
- Do not in any way attempt to tamper with or alter the illuminator; doing so voids the warranty, and may damage the system. This product does not contain consumer serviceable components other than the replaceable lamp and the fuses. Contact PerkinElmer for any other required service or repairs.
- Ensure that the cooling vents in the controller case are not blocked.

Warnings:
- Hg-LAMP CONTAINS MERCURY. Handle, maintain and dispose of the lamp in accordance with local Disposal Laws.
- Before replacing a fuse, DISCONNECT THE ILLUMINATOR FROM THE POWER SUPPLY.
- Eye damage may result from directly viewing the light produced by the lamp used in the illuminator.
- Always make sure the light guide is properly attached to the illuminator and inserted into the collimator, and that the collimator is firmly attached to the microscope before turning on the power to the unit.
- The illuminator has built-in protection features to avoid unintentional exposure to UV radiation. Do not attempt to defeat the protection features.

Caution:
- Never look into the emitting end of a light guide. The light could severely damage the cornea and retina of the eye if the light is observed directly.
- Appropriate eye shielding must be used at all times; clothing should be used to protect exposed skin.
- Never place the end of an emitting light guide near skin as this may result in burning and damage to the skin.
- Never place the end of an emitting light guide near a flammable substrate, as sufficient power is emitted from the light guide to ignite flammable substances.
- When turned on, the illuminator should be attended at all times by a qualified operator. Do not leave

the illumination lamp on and unattended longer than the amount of time required to complete operation.

1.6 Table of Symbols

The table below contains symbols that identify particularly important information and alert you to the presence of hazards. These symbols may appear in this manual and/or on the product it describes.

Warning: Read instructions to determine possible hazard.
Caution: Read these operating instructions fully before use, and pay particular attention to sections containing this symbol.

Warning: Surface may be Hot.

Warning: UV Output

Danger: Electrical Shock hazard.

AC Input

DC Input

USB Ports

Connects to Computer

Connects to Foot Pedal. This port is not currently used. Do not plug other devices into this port.

Connects to Microphone. This port is not currently used. Do not plug other devices into this port.

1.7 Technical Support

If you experience any difficulty setting up, operating, or maintaining the Mantra system, please contact your PerkinElmer representative. Office hours are 8:00 a.m. to 8:00 p.m. (Eastern Standard Time), Monday through Friday.

- Telephone: 800-762-4000 or +1 203-925-4602
- Fax: +1 203-944-4904
- Email: global.techsupport@perkinelmer.com.

To contact a local PerkinElmer representative outside the United States, go to:
- Website: www.perkinelmer.com

1.8 About This Manual

This manual describes the use and functionality of the PerkinElmer Mantra Quantitative Pathology Imaging System and the Mantra Snap 1.0.0 software. Operating instructions, functional descriptions, troubleshooting, illustrations, and other relevant information are contained in this manual.

Design Change Disclaimer

Due to design changes and product improvements, information in this manual is subject to change without notice. PerkinElmer reserves the right to change product design at any time without notice to anyone, which may subsequently affect the content of this manual. PerkinElmer makes every reasonable effort to ensure that this User's Manual is up to date and corresponds with the shipped Mantra Quantitative Pathology System.

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1.9 CE Testing and Certification

The Mantra Quantitative Pathology System has been tested by an independent CE testing facility, and bears the appropriate CE mark.

Note: Changes or modifications to this equipment not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Remarque: Tout changement ou modification apporté à cet instrument non expressément approuvé par l'entité responsable de la conformité peut annuler l'autorisation d'opérer l'appareil accordée à l'utilisateur.

Contact PerkinElmer at global.techsupport@perkinelmer.com for more information.

2 Imaging With Mantra

The best way to learn how to use the Mantra workstation is to follow this portion of the user's manual. Imaging With Mantra 13 will guide you through taking a brightfield image and taking a fluorescence image. Your Mantra system comes with inForm, PerkinElmer's advanced image analysis program. The Mantra Snap program can be configured to automatically send each image to inForm. inForm can be set up to open your images and even to automatically process them using a predetermined 'algorithm' or image-processing routine can be done from within the Mantra Snap program.

You may wish to have the inForm user's manual available, because if you would like to try using this automatic-transfer feature, the following exercises can be continued in the inForm user's manual in *Appendix B: Auto-Processing Data Received from Mantra.*

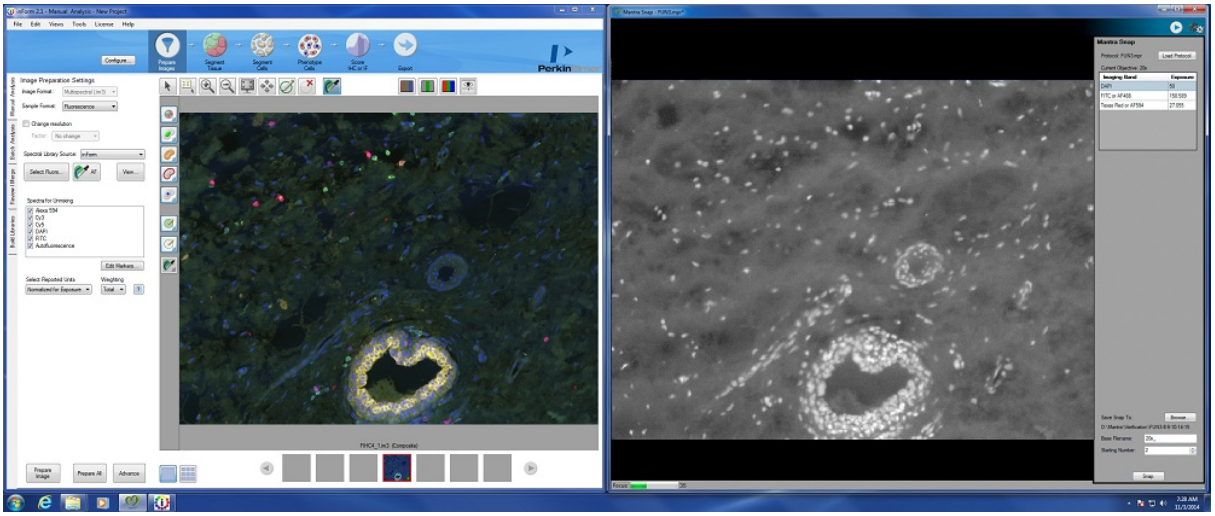

Figure 2. The Mantra system comes with the Mantra Snap software program for image acquisition and the inForm Tissue Finder advanced image analysis software program for trainable image analysis that can be easily automated.

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2.1 Hardware Setup

The Mantra system is normally installed and configured for the first time by a PerkinElmer technician. Training and Service packages beyond initial set-up and training are available at nominal cost. In this section, we will simply confirm that the Mantra hardware is set up properly before use.

1. Check that the Power and USB cables, shown in Figure 3 (far left), are plugged into the connectors on the back of the Mantra Imaging Module 6
2. Check that the USB cable for the computer from the Interface Panel 7, identified in Figure 2, on the left side of the Mantra instrument is plugged into the computer
3. Check that the Power cable from the Interface Panel is plugged into a surge-protected power outlet

Figure 3. Power and USB cables plugged into the back of the Mantra imaging module (far left); the location of the Mantra Power switch outlined in red (middle left); objectives in their numbered turret positions (middle right); and epi-fluorescence filter cubes in their designated, numbered locations (far right).

If the Mantra system is not already on, switch the power switch, shown in Figure 3 (middle left), located on the lower left of the black panel on the left side of the main instrument body, to the ON position.

The default objectives in the motorized objective turret 7, shown in Figure 3 (middle right), are 4X, 10X, and 20X. A 40X objective is optional. The epi-fluorescence excitation 'cubes' are located in their own motorized turret 7 between the objectives and the eyepieces.

IMPORTANT: The objectives and the epi-fluorescence filter cubes are installed in specific, numbered locations in their respective turrets. Do not move them into different locations or replace them with other objectives or epi-fluorescence filter cubes unless instructed to do so. The default positions associated with each epi-fluorescence filter are labeled on the epi-fluorescence filter turret cover shown in Figure 3 (far right):

Position 1 – BF, brightfield (no filter cube present)

Position 2 – DAPI

Position 3 – FITC

Position 4 – CY3

Position 5 – TXRED, Texas Red

Position 6 – CY5

Position 7 – CY7, if present (optional)

Position 8 – QUAD, quad-band filter for visualization through the eyepieces (optional)

If you wish to perform fluorescence imaging, the Mantra Fluorescence Illuminator 62, shown below in Figure 4, should be connected to the rear of the Mantra instrument by a liquid light guide. The

illuminator's power cable should be connected to a power outlet. Switch the power switch located on the lower left front of the Mantra Fluorescence Illuminator to the ON position.

The Mantra Fluorescence Illuminator is a 200W module that provides fluorescence epi-illumination of slides on the Mantra microscope stage. It has 6 levels of illumination (0% , 10%, 25%, 50%, 75%, and 100%) using a self-aligning, temperature-controlled 200W metal halide bulb. If you need to replace the bulb, see Appendix C: Mantra Fluorescence Illuminator: Setup and Maintenance 62

IMPORTANT: A minimum of 4 inches (100mm) clearance around the Mantra Fluorescence Illuminator should be maintained at all times. Equipment and objects should be placed in such a manner that the power switch and disconnecting devices are accessible at all times.

Although the illuminator will reach 70% output in 1—5 minutes, allow 30 minutes for it to reach operational temperature.

WARNING: Do not switch the unit off within 5 minutes of switching it on. Not complying with this warning may result in damage to the bulb.

After switching the unit off, allow the bulb to cool for at least 30 minutes before changing the bulb or switching the unit back on. Failure to do so is likely to result in damage to the bulb.

Figure 4. Switch on the Mantra Fluorescence Illuminator if you wish to perform fluorescence imaging. The illuminator should be allowed 30 minutes to reach operational temperature before imaging begins.

See Appendix C: Mantra Fluorescence Illuminator: Setup and Maintenance 62 for a list of warnings that might be displayed on the illuminator's front panel display and that have corresponding actions.

You can adjust the Mantra microscope hardware, including the slide stage, focusing controls, and eyepieces by referring to Appendix A: Mantra Hardware Setup 50 for instructions. If you have installed a new lens or epi-fluorescence filter, need to reconfigure your computer interface, or want to Koehler-align the microscope optics, refer to the section that describes the Mantra Hardware Setup Program.

2.2 Installing and Starting the Mantra Snap Software

The Mantra Snap software requires an activated software license to run. The software was installed and activated on the Mantra computer at the factory.

If you need to re-install or re-activate the Mantra Snap software, please contact PerkinElmer Service & Support at US Toll-Free 1-800-762-4000 or your local PerkinElmer Custom Care Center or authorized distributor. You may download the most recent version of the software by visiting us at: www.perkinelmer.com

See Appendix B: Installing the Mantra Software 61 for a detailed description of the software installation and activation processes.

IMPORTANT: The Mantra instrument must be attached to your computer and powered up in order to run the Mantra Snap software and acquire images. If the Mantra ever loses power, Mantra Snap will shut down.

Recovering a Lost Seat License

Mantra Snap licenses are not recoverable. If your Mantra Snap seat license is damaged or inadvertently lost (e.g. your hard drive crashes), contact Technical Support 11 for assistance. Please have your Mantra system serial number (5 digits in length) and/or your Mantra Snap license number (20 digits in length) ready.

If you wish to capture images using the Mantra instrument, power up the Mantra system as described in the previous section and start your computer. When Windows has finished booting, start the Mantra Snap program by double-clicking on the Mantra Snap icon on the desktop, shown below in Figure 5, or go to

Start >> All Programs >> PerkinElmer >> Mantra >> 1.0.0 >> Mantra 1.0.0

Figure 5 Mantra Snap Desktop Icon

2.3 Understanding the Mantra Snap Work Area

The Mantra Snap program is designed to help you capture the images you want with as little effort as possible. You can keep your hands on the controls built into the Mantra instrument instead of making multiple clicks on a mouse button and looking back at the computer monitor. You can even configure the Mantra Snap software to automatically send your images to the included inForm software for processing while you take more pictures.

Shown below in Figure 6, are the important interface elements of the Mantra Snap program:

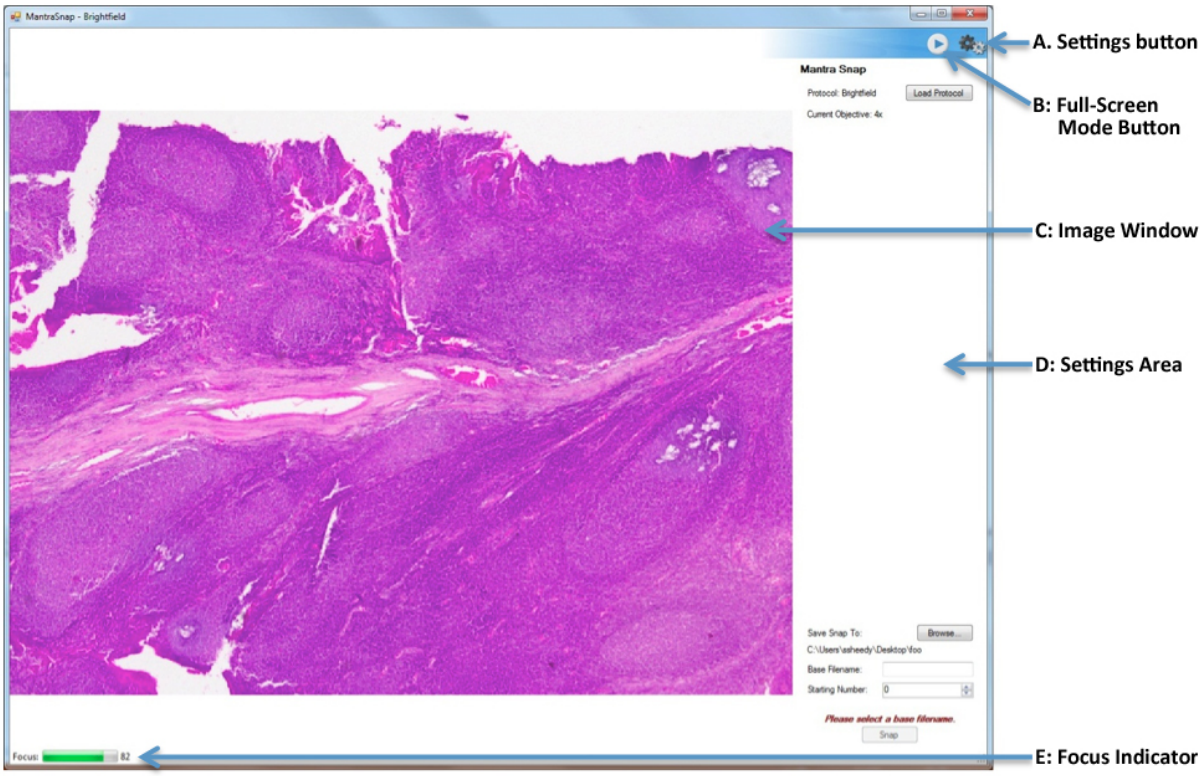

Figure 6 Mantra Snap brightfield interface is shown above. Note the large Image Window with the Settings Area on the right. Different settings can be selected using the Settings Button in the upper right side of the window, just below the red Close Window button.

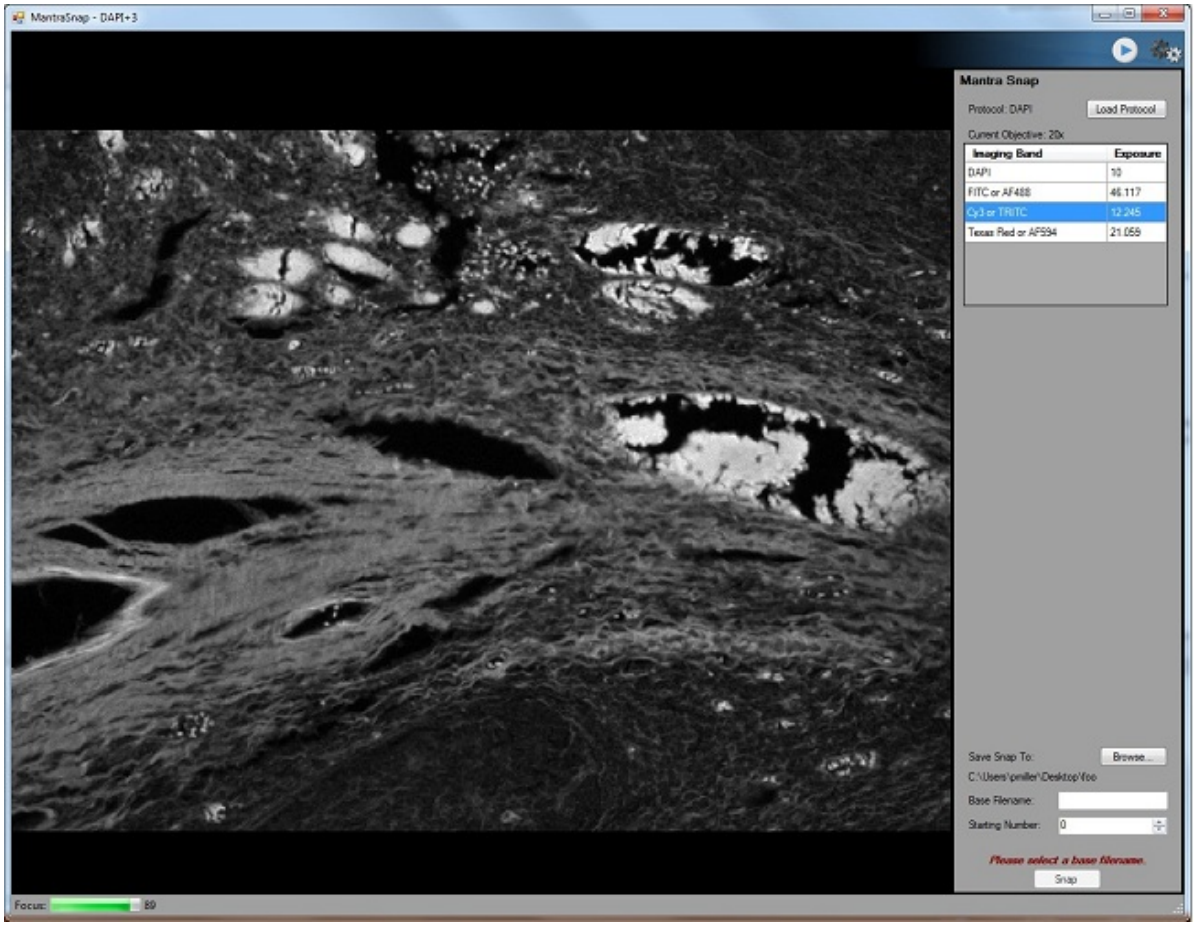

Figure 7. Mantra Snap fluorescence interface is shown above. Note that the work area has a more subdued, darker theme than the brightfield interface. This darker theme reduces the amount of stray light in your laboratory if you are intentionally working in reduced-lighting conditions. There are also some additional menu items that are unique to taking pictures in fluorescence mode.

The Mantra Snap program in brightfield mode has a white background, while the Mantra Snap program in fluorescence mode has a dark gray background. The darker theme, shown in Figure 7, reduces light scatter from the computer monitor when the imaging system is being used in a darkened laboratory area. Both Mantra Snap interface themes share the same basic elements:

A: Settings Button: allows you to choose, in a drop-down menu, which settings you would like to change. The list of settings for Brightfield and for Fluorescence is slightly different, as shown in Figures 8 and 9.

B: Full-Screen Mode Button: toggles between displaying just the live image or showing the live image and all available tools, such as the Settings Area and Focus Indicator.

C: Image Window: the large window in the work area where the live image is displayed

D: Settings Area: the vertical area to the right of the Image Window where Mantra Snap settings are displayed and can be edited

E: Focus Indicator: green bar that changes length and a number that increases or decreases based on how sharp the image in the Image Window

appears

Settings Menu

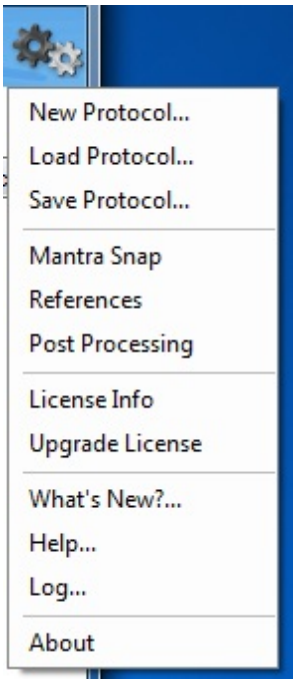

Figure 8. Mantra Snap drop-down menu settings for brightfield.

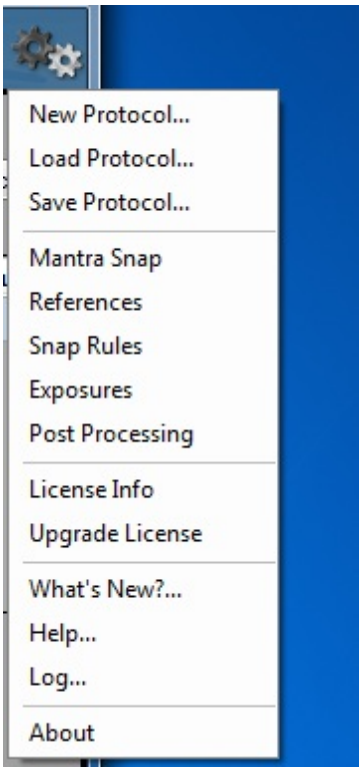

Figure 9. Mantra Snap drop-down menu settings for fluorescence.

Drop-down menu settings (as shown in Figures 8 and 9) vary slightly for brightfield and fluorescence imaging:

- **New Protocol…**: creates a new protocol for which you will need to choose settings. CTRL+N will bring up the new protocol dialog, where you can choose to create a brightfield or fluorescence protocol.
- **Load Protocol…**: allows you to load an existing protocol (e.g. the default brightfield or fluorescence protocols provided by the factory, or a protocol that you have created or modified). Existing protocols can be opened, modified, and then saved under different names to create customized, new protocols. CTRL+O will bring up the load protocol dialog.
- **Save Protocol…**: saves a protocol that you have created or modified. CTRL+S will bring up the save protocol dialog.
- **Mantra Snap**: the main settings window for taking pictures using the Mantra system. From here you can load protocols, specify a location to save your images, specify a base filename and starting number for your image files, and take pictures using the software Snap button or the Snap button on the Mantra instrument itself.
- **References**: allows you take reference images and review them if necessary

- **Snap Rules** [Fluorescence mode only]: lets you choose which fluorophores you want to image and the order in which the images are taken of each one.
- **Exposures** [Fluorescence mode only]: lets you set the exposure (in milliseconds) that you wish to use for each fluorophore.
- **Post Processing**: lets you decide if you would like your images automatically transferred to the included inForm advanced image analysis program or a Windows batch process file run.
- **License Info**: displays your Mantra Snap software licensing information
- **Upgrade License**: allows you to upgrade to additional functionality (i.e. pay for additional features). Free incremental upgrades are handled using the standard installation procedure and do not use this.
- **What's New?**: find out which features in this software version are new and obtain a description of each new feature.
- **Help…**: Access to the online help feature, including this document
- **Log…**: access to the log file that could be useful for record-keeping or troubleshooting
- **About**: information about this software release version.

2.4 Taking Brightfield Images: A Practice Exercise

This section will guide you through taking brightfield images using the Mantra imaging system. The section that follows this one describes taking fluorescence images. Skip to that section if you want to take fluorescence images instead.

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- Taking a Brightfield Image 24
- Processing a Brightfield Image in inForm 26

2.4.1 Configuring Mantra for Brightfield Imaging

Make sure that the Mantra hardware is properly configured and powered up 14. Then turn on the computer and wait until Windows has finished booting up. Launch the Mantra Snap software by double-clicking on the icon on the desktop or by going to C:\Program Files\PerkinElmer\Mantra\1.0.0 \MantraSnap.exe.

In the Mantra Snap program, load the default brightfield protocol by clicking on the Settings button 19 in the upper right of the Mantra Snap program window and selecting **Load Protocol…** from the drop-down menu. You will be automatically taken to C:\Users\Public\PerkinElmer\Mantra\Protocols.

Select Brightfield.mpr

> *IMPORTANT: Do not move or rename the Protocols folder. You may create folders within, but do not move or rename this 'root' folder for loading and saving protocols. You can save new protocols, but only within the Protocols folder or within folders located inside the Protocols folder.*

Once the default Brightfield protocol is loaded, you will be automatically placed into brightfield mode and prompted to take your reference images if this is the first time you have used the system. You might also be prompted to slide the Beam Block, shown in Figure 10 , out of the way if it is covering the brightfield illuminator.

Figure 10. The Beam Block (the plastic cover shown in the red box) slides over the brightfield illuminator when you are using the Mantra system to take fluorescence image. The Mantra Snap program will prompt you to slide it into or out of place when necessary.

Taking References

You can skip ahead past this step if reference images have already been taken.

Follow the directions in the Mantra Snap program to take your reference images. The Mantra system will select different objectives, adjust exposures by itself, and prompt you to take actions such as to change the setting of the trinocular optical path switch or move the slide to a new location using the stage controls.

When the references have been taken, click **Done** and you will be taken back to your initial settings (your previously selected objective and illumination setting).

NOTE: Reference images help produce optimal images no matter what objective is used. They compensate for small optical imperfections that are a normal part of any optical system. You will need to have a clean microscope slide available in order to follow the directions for taking your reference images. You will need to take a set of reference images the first time you start using the system, but not necessarily for every imaging session.

As the lamp ages or if you notice that there are unwanted non-uniformities in the images, you can take new reference images by choosing References in the drop-down menu. Taking new reference images can take 5—10 minutes, but it is not an especially challenging task. We recommend that your lab decide upon a procedure that specifies when new references should be taken.

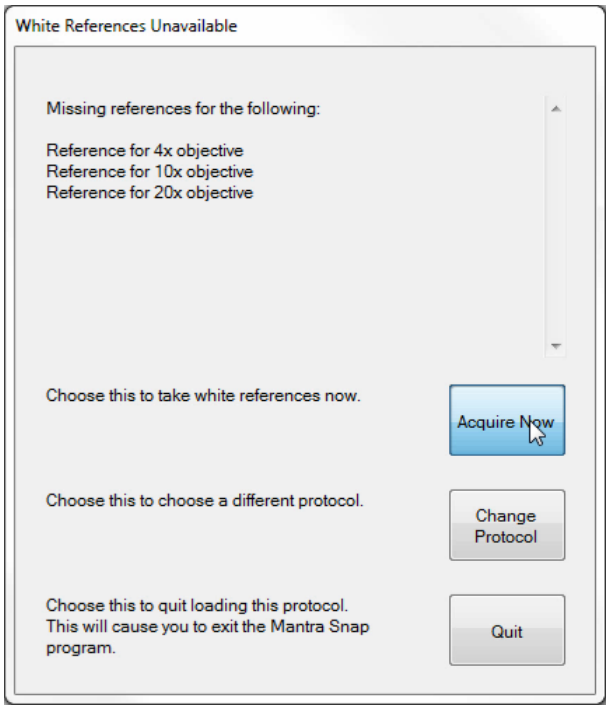

White References Unavailable
Missing references for the following:
Reference for 4x objective
Reference for 10x objective
Reference for 20x objective
Choose this to take white references now.
Acquire Now
Choose this to choose a different protocol.
Change Protocol
Choose this to quit loading this protocol.
This will cause you to exit the Mantra Snap program.
Quit

Figure 11. Taking reference images is made easy by following the directions in the Mantra Snap program. Good reference images ensure the high-quality images from your slides. You do not have to take reference images every time you use the Mantra, but you certainly must take them the first time you use it.

If you have already taken references, you can view them using the references editor. References are stored for two trinocular positions; Camera Only and Shared. Adjust your trinocular to one of those two positions, then click **View References** to see the reference images for that position.

If you would like to retake your references, click **Take References.**

Click **Done** when you are finished, or use the menu to select another editor.

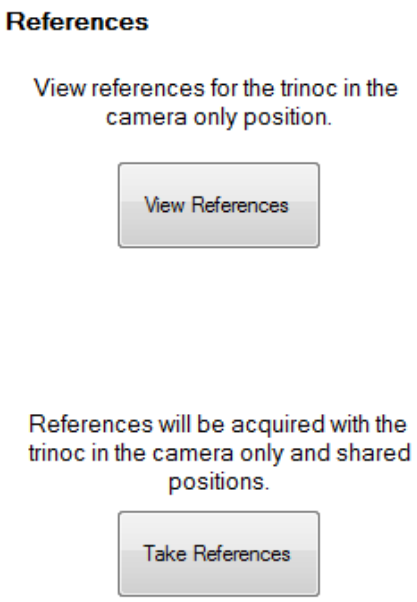

References
View references for the trinoc in the camera only position.
View References
References will be acquired with the trinoc in the camera only and shared positions.
Take References

Figure 12 Brightfield References Editor

Adjusting the Lamp

Locate a suitable brightfield slide (e.g. H&E or hematoxylin & DAB) and place it onto the stage below the objectives. You may set the trinocular optical path switch so that some or all of the light goes to the eyepieces. You will see a live streaming image of the slide on the monitor.

Use the Objective rocker switch on the left side of the lower front control panel to choose the objective, and adjust the illumination level using the knob on the right side of the panel, as shown in Figure 13. The level that you set for each objective is automatically remembered by the Mantra system. So once you have adjusted the illumination level for each objective, switching from one to another does not require that you re-adjust the level by hand.

Figure 13. You can use the Objective rocker switch to select the objective you want. In brightfield mode, the blue light above the FL (fluorescence) label remains off and the Epi-Filter switch is deactivated. You can set the illumination level that is comfortable for you through the eyepieces using the BF Lamp knob on the right.

2.4.2 Taking a Brightfield Image

Locating Your Sample

With your sample on the stage, locate a portion of the slide that you wish to image. You may use the Stage Control knob, shown in Figure 14, to move the stage or handle the slide directly. The knob is normally located to the right of the stage, next to one of the large focusing knobs. You can have the stage control knob, as well as the main focusing knob moved to the opposite side. Contact PerkinElmer's Service and Support team for details on performing the procedure.

Figure 14. Use the Stage Control knob located by default on the right side of the Mantra instrument to move the microscope stage along the X- and Y-axes.

Destination Folder

Select a destination folder for your images by clicking on the Save Snap To: **Browse…** button at the bottom of the Mantra Snap Settings area to the right of the live image. The default location is D:\Data\MantraSnap.

NOTE: the Mantra computer comes with two hard disk drives, one for the Windows operating system and the Mantra Snap and inForm software (C: drive) and one for your data (D: drive). PerkinElmer recommends saving your data to the D: drive and making back-up copies whenever possible to avoid data loss in case your hard disk drive ever fails. Your institution will almost certainly have a network to allow access to larger storage areas for your data. The Mantra computer comes with a high-speed Gigabit Ethernet port for this purpose.

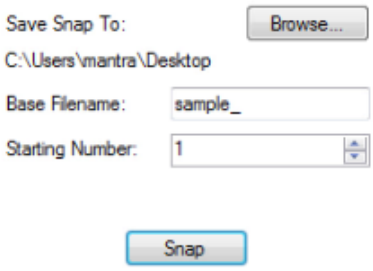


Figure 15. Mantra Snap Controls

Base Filename

Enter a new name in the **Base Filename** text box and choose a **Starting Number**. New images will be placed into a folder named after the base filename that you have entered. Those images will be similarly named with the base filename and a number, beginning with the starting number you have entered. The default starting number is 1 (one). The file format suffix is PerkinElmer's .IM3. This file format preserves relevant information from the Mantra system including but not limited to the imaging mode, magnification, exposure times, stains that were present on the slide, and creation date.

So, if you entered a Base Filename of 'My IHC Slide' and chose a Starting Number of '5', the first image

you take will be named ‘My IHC Slide 5’ (or ‘My IHC Slide 5.im3’ if you have chosen to have Windows show file-type extensions) and placed into a folder named ‘My IHC Slide’. The next image you take will be named ‘My IHC Slide 6’ and placed into the same folder. You may choose any starting number, but you will be warned if your choice inadvertently results in a file with the same name in the same location as an existing file. You will be given a choice to overwrite the existing file or to select a different filename.

Taking an Image

Focus the image using one of the focus control knobs on the Mantra microscope body. You can see the slide in real time on the monitor. The green bar of the **Focus Indicator** in the lower left of the Mantra Snap program grows longer as the image displayed is sharper and becomes shorter as the image displayed is less sharp. The green bar may not extend to the full length of the indicator’s space. So simply strive for the longest green bar and the highest number possible.

Figure 16. The Mantra ‘Snap’ button to take pictures is located on the handgrip on the left side of the Mantra instrument base. In the picture above, the Snap button is located under the user’s left thumb. The Objective rocker switch to change objectives is located under the user’s right thumb.

When you are ready, push the Snap button on the handgrip located to the left of the Mantra lower front control panel, shown in Figure 16, or click on the **Snap** button at the bottom right of the Mantra Snap program, as seen in Figure 15. You may observe the slide through the eyepieces or watch the LCD monitor while snapping your images.

> *NOTE: If you hold down the Alt key on your keyboard while clicking on the Snap button in the Mantra Snap program, the button changes from Snap to Snap Color and you will simply save a color (TIFF) image instead of a full multispectral .IM3 file.*

The Mantra Snap program will warn you if you have inadvertently set the trinocular optical path switch directing all of the light to the eyepieces (and none to the camera) if you try to snap a picture when in that mode. You can take pictures with either some or all of the light directed to the camera.

If you wish, you can click on the **Full-Screen Mode** button and the image will fill the Mantra Snap window, hiding the Settings Area. This can be very useful if you are only using the hardware controls and wish to have as large an image as possible on the screen.

As previously described, if you push the switch to change your objective, as shown in Figure 16., Mantra Snap remembers the illumination level and adjusts it for you. So if you have optimized the illumination level for your eyes and/or camera while at the 4x setting and then decide to change to the 10x or 20x setting, you do not have to re-adjust the illumination level when the new objective clicks into place—Mantra does that for you. Of course, you can adjust the illumination level at any time (Mantra will not override your choice). You can see a representation of the illumination level by observing the blue dots that surround the knob that controls the illumination level on the right side of the lower front control panel, as shown in Figure 13.

IMPORTANT: While Mantra can adjust the illumination level from objective to objective, it does not adjust the focus setting. So you might need to fine-tune the focus when switching objectives.

NOTE: The Mantra's Shutter *button is disabled when you are in Brightfield mode. If you push on the button, located below the Snap button, you will hear a clicking sound, but the shutter setting for the fluorescence illumination system will not change.*

Take a few more images to familiarize yourself with the Snap process. Next, you could set up a Post-Process procedure 46 by going to the Settings button and choosing **Post Processing**. You can refer to the inForm User's Manual, version 2.1.0 or later, for additional details on the program's capabilities and features.

In order to save you from flipping back and forth amongst the two separate user's manuals for this particular exercise, the directions in Processing a Brightfield Image in inForm 26 pertain to the inForm Advanced Image Analysis software program that comes with each Mantra system.

2.4.3 Processing a Brightfield Image in inForm

Under normal circumstances, you would capture images from single-stained slides in order to build up a 'library' of spectra (precise colors that are really unique multispectral profiles of your stains). PerkinElmer calls this a 'Stain Store' of the stains and fluorophores that you would see in the normal course of your research.

To help get you started, PerkinElmer includes some commonly used and characterized fluorescence spectra, referred to as 'synthetic' stains because they were not captured and characterized on your particular Mantra system. However, because of the variability of stains and the staining process in brightfield IHC, there are no synthetic spectra provided for brightfield microscopy.

Nevertheless, with an H&E or hematoxylin & DAB slide that features areas of relatively pure staining of one or both stains, you can capture and save representative spectra that will be useful for instructive purposes only.

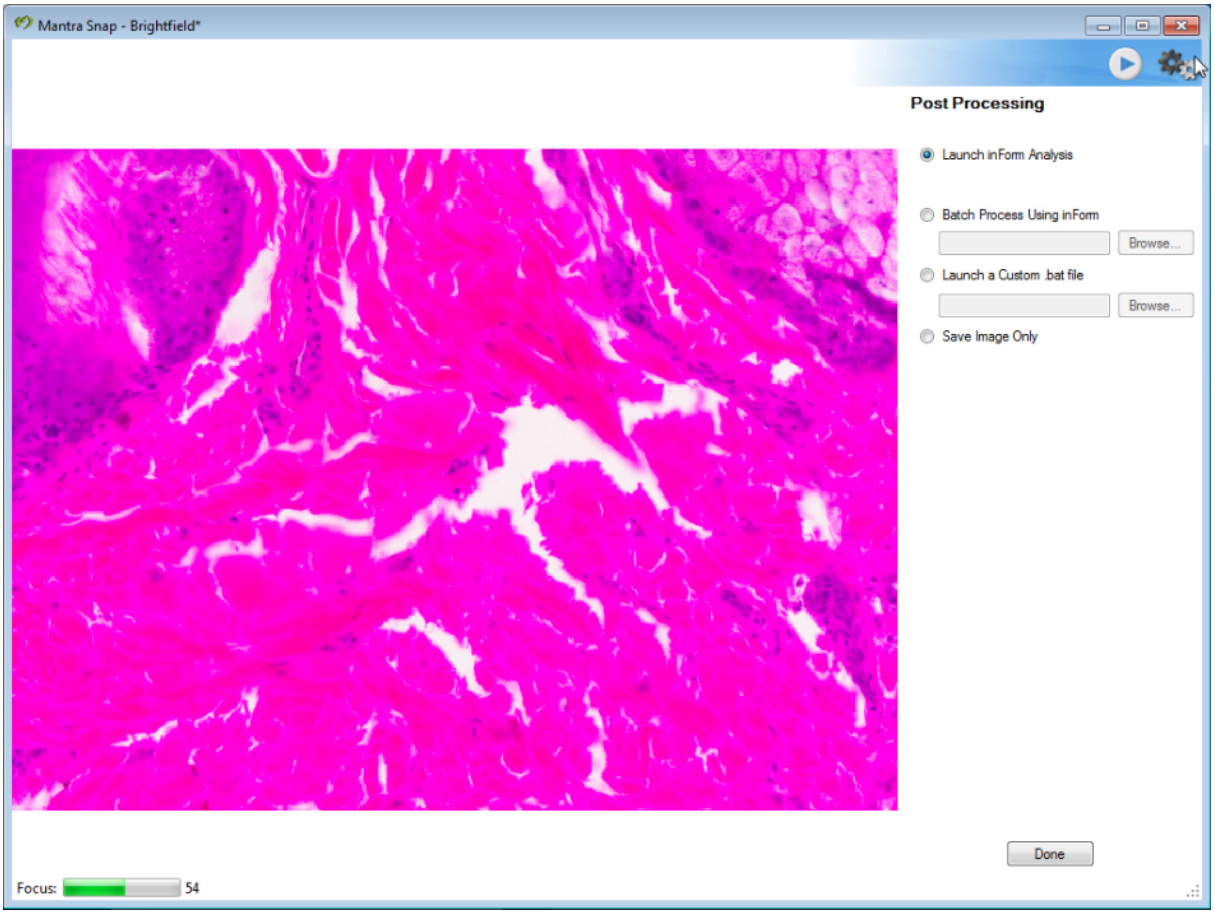


Figure 17. You can configure Mantra Snap to simply save your images, or you can configure it to automatically launch the inForm Advanced Image Analysis Software program, perform a batch process using inForm, or even launch a custom Windows .bat file.

In the Post Processing settings editor, seen in Figure 17, above, select **Launch inForm Analysis**. Locate an area on your sample slide (presuming that you have a properly stained H&E slide for purposes of instruction here) that features a large area of nearly pure eosin and snap a picture using the Mantra Snap program. In a moment the inForm program window will appear as shown in Figure 18, below.

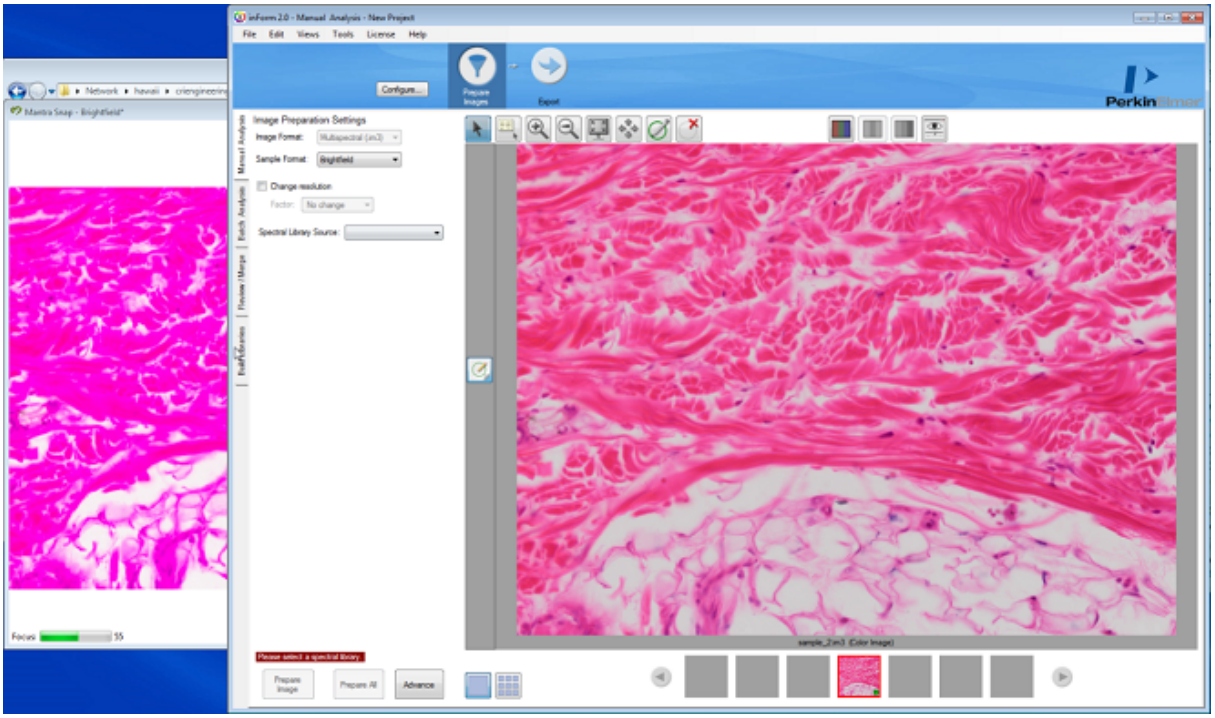


Figure 18. When you select Launch inForm Analysis in the Mantra Snap program, the inForm program window will appear as shown, with the image that you snapped loaded into the Image Display Area of the inForm workspace. You can see the Mantra Snap program behind and to the left of the inForm program window.

Click the lower **Build Libraries** tab in the far left of the inForm program. You will be asked to save the current project. You should click **No** and then load the image that you just captured by clicking on the Load Image: **Browse…** button and selecting the saved image.

When the image appears in the inForm work area, it will be automatically detected and classified as a brightfield image (see the grayed-out Sample Format: 'Brightfield'). Choose **Stain: Eosin** from the drop-down menu.

Most likely you will receive a message that more than one stain was detected in your particular specimen. This is normal if the slide contains more than one stain, in our example a large amount of eosin and a very small amount of hematoxylin.

If that is the case, click on the **Draw Processing Regions** button at the top of the work area and draw one or two regions around areas that feature just the eosin and little if any hematoxylin.

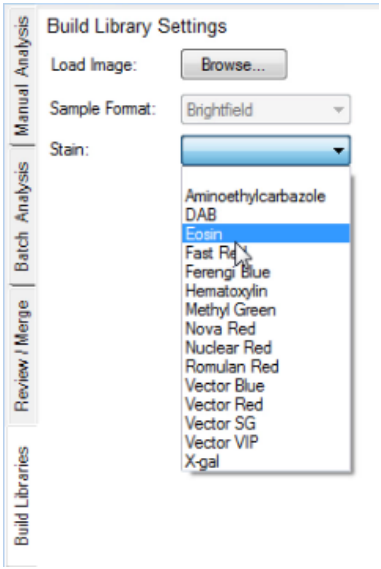


Figure 19. You can choose from a drop-down menu to identify the pure stain present in your sample.

Click **Extract.**

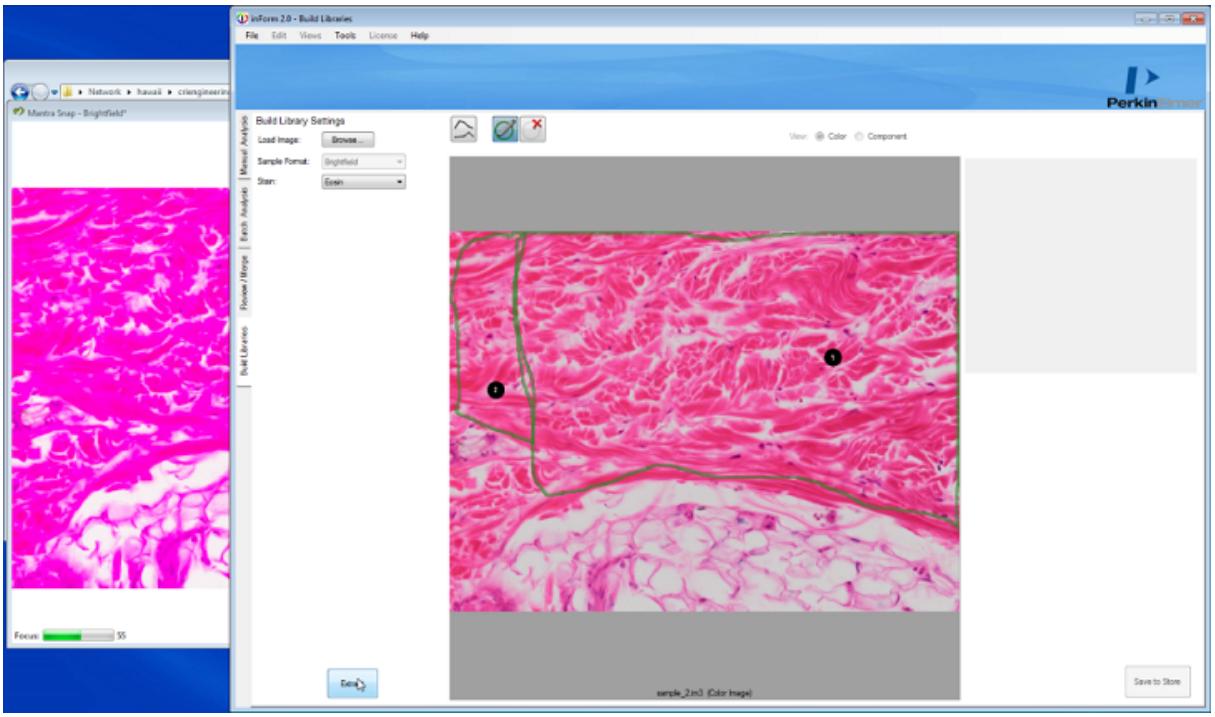


Figure 20. You can select areas in your slide than contain pure or nearly pure signal, in this case eosin, if you do not have samples of tissue prepared with only the single stains.

The inForm program will extract the pure signal, or a signal that is very close to a pure signal: in this case representing eosin. **Save** the stain in a Group with a name of your own choosing. Try to pick a name that differentiates this stain as a test, rather than a stain from an image and spectra gathered from a real, pure single-stained sample.

You can now repeat the above steps to extract the spectra for the second stain on your slide, or if you have a previously saved image with, in this case pure hematoxylin, you can open the Mantra .IM3 file. An example of this is shown below.

Select **Stain: Hematoxylin** from the drop-down menu and then click **Extract**.

Save the pure spectra from the second stain just as you did with

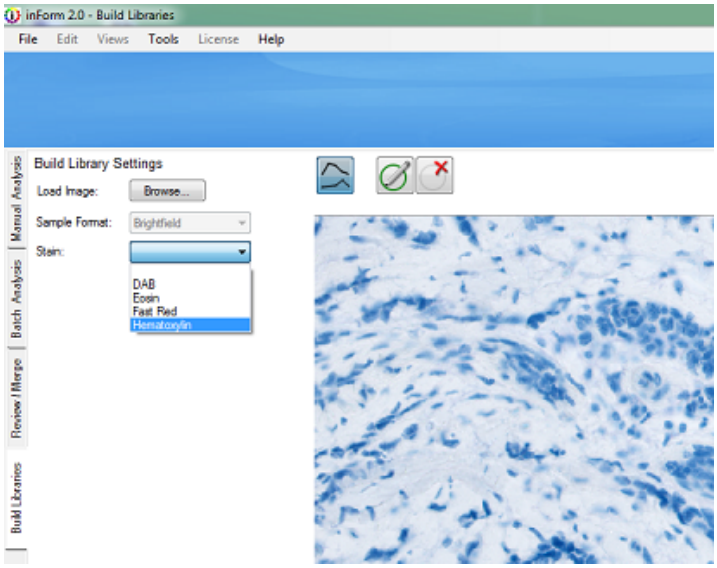


Figure 21. For your second stain, choose from the drop-down menu. If this is from a real, single-stained slide, inForm will be able to easily extract the pure spectra from it.

the first. Then click on the **Manual Analysis** tab in the upper left side of the inForm work area.

Click on the **Select Stains** button. When the Select Stains window opens, click the checkboxes representing the stains that are present on your sample slide and then click **OK**.

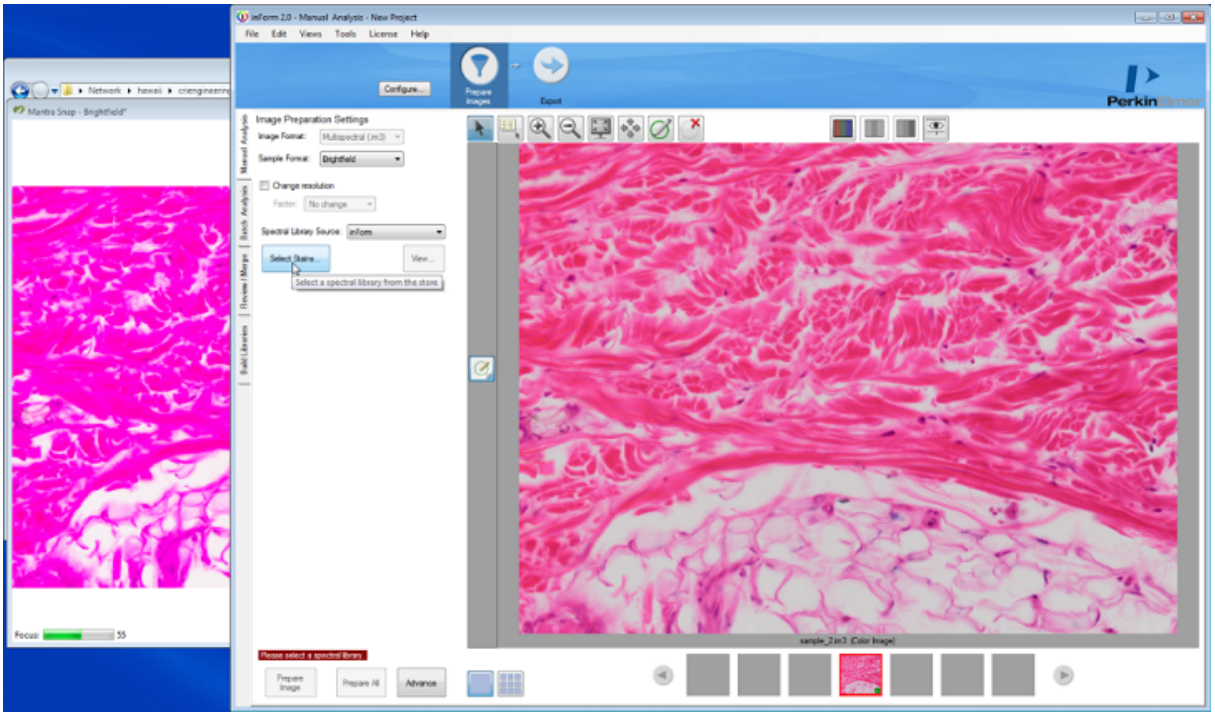


Figure 22. Back to the Manual Analysis tab in inForm where you can now click on the Select Stains button to identify the stains for which you previously saved the spectra.

New information and options will appear below the Select Stains button, including a list of the Spectra that will be used for 'Unmixing', spectrally separating the two stains from another in your image. Click **Prepare Image** to unmix the image.

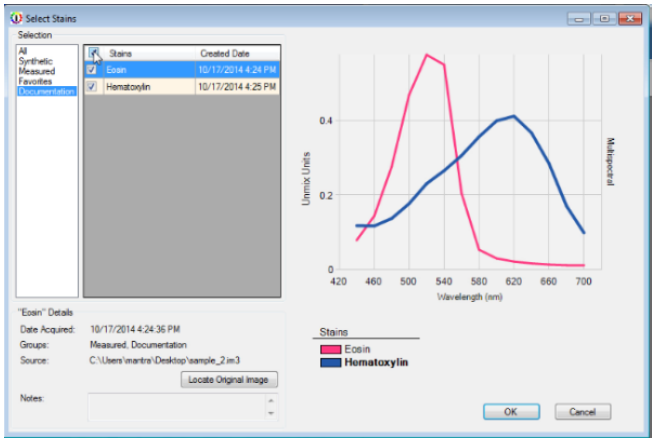


Figure 23. You can now select the spectra of the two stains that are present in your sample slide.

Once the image has been unmixed, you can use the **Pathology Views** button to show just the hematoxylin or just the eosin. You can also show the image as it would appear in fluorescence.

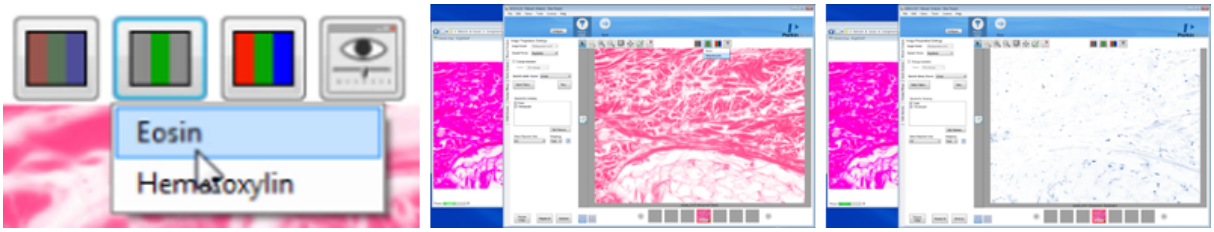


Figure 24. The Pathology Views button (left) allows you to select and view the individual stains, in this case eosin (middle) and hematoxylin (right).

Now that you have successfully chosen stains and unmixed an image, you can return to the Mantra Snap program and continue to take images. As long as the inForm program window is open, the new images will be processed just like the first image that you worked on. Of course, if you switch to a different slide that was prepared with different staining or stains, your results will not be consistent with the first slide.

2.5 Taking Fluorescence Images: A Practice Exercise

This section will guide you through taking fluorescence images using the Mantra imaging system. Go to the previous section if you wish to take brightfield IHC images instead.

- Configuring Mantra for Fluorescence Imaging 31
- Taking a Fluorescence Image 38
- Processing a Fluorescence Image in inForm 41

2.5.1 Configuring Mantra for Fluorescence Imaging

Make sure that the Mantra hardware, including the fluorescence illuminator, is properly configured and powered up 14. Then turn on the computer and wait until Windows has finished booting up. Launch the Mantra Snap software by double-clicking on the icon on the desktop or by going to C:\Program Files \PerkinElmer\Mantra\1.0.0\MantraSnap.exe.

If you are working in a laboratory with subdued lighting, you can change the wallpaper on your computer monitor to a darker theme.

Go to C:\Program Files\PerkinElmer\Mantra\1.0.0\, right-click on PKI_AltBackground.jpg and choose Set as Desktop Background.

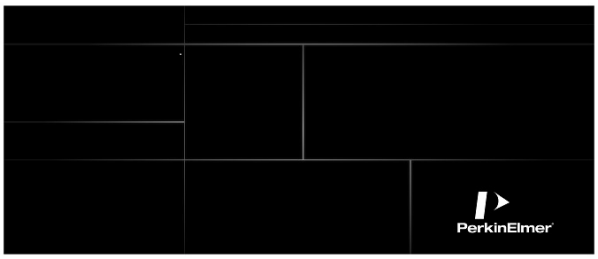


Figure 25. You can select the alternative, darker wallpaper for fluorescence imaging in a lab with subdued lighting.

Load the default fluorescence protocol by clicking on the Settings button in the upper right of the Mantra Snap program window and select **Load Protocol**… You will be automatically taken to C:\Users\Public \PerkinElmer\Mantra\Protocols.

Select DAPI.mpr

IMPORTANT: Do not move or rename the Protocols folder. You may create folders within, but do not

move or rename this 'root' folder for loading and saving protocols. You can save new protocols, but only within the Protocols folder or within folders located inside the Protocols folder.

Once the fluorescence protocol has been loaded, you will be automatically placed into fluorescence mode and prompted to take your reference images if this is the first time you have used the system. The presence of a blue LED dot located above the letters FL on the lower front control panel indicates that you are in fluorescence mode.

NOTE: The Mantra Snap program's fluorescence mode has a dark gray background. The darker theme reduces light scatter from the computer monitor when the imaging system is being used in a darkened laboratory area. This also makes it easier to differentiate between the fluorescence mode and the brightfield mode with its bright white theme.

If it is not already in the proper position, you will be directed to slide the Beam Block, shown in Figure 26 over the brightfield illuminator located below the condenser. The brightfield illuminator is not needed for fluorescence imaging and the shield/cover reduces the amount of stray light entering the objective, improving image quality. In fact, Mantra will not let you proceed with taking fluorescence images until you have slid the Beam Block into place, covering the brightfield illuminator.

Figure 26. The Beam Block (the plastic cover shown in the red box) slides over the brightfield illuminator when you are using the Mantra system to take fluorescence image. The Mantra Snap program will prompt you to slide it into or out of place when necessary.

Taking References

You can skip ahead past the steps describing how to take fluorescence reference images if they have already been taken.

Locate the blue fluorescence reference slide, included with your Mantra system. Follow the directions to take your reference images. The Mantra system will select different objectives, select the epi-fluorescence filters by itself, and prompt you to take actions such as to change the setting of the trinocular optical path switch or move the slide to a new location on the stage.

When the fluorescence references have been taken, click **Done**.

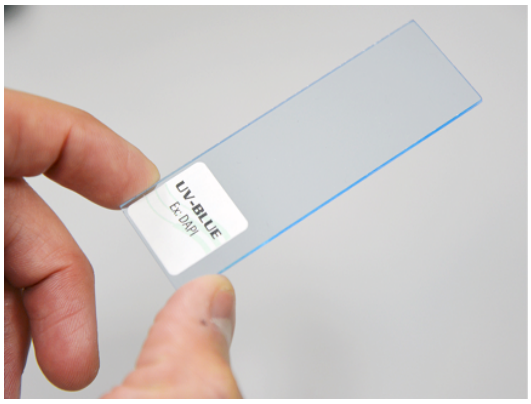


Figure 27. The Mantra fluorescence reference slide is blue in color and is used for taking your reference images when in fluorescence mode. Store it in a safe location. If you damage or misplace it, contact PerkinElmer to obtain a replacement slide.

NOTE: Reference images help produce optimal images no matter what objective is used. They compensate for small optical imperfections that are a normal part of any optical system. You will need to take a set of reference images the first time you start using the system, but not necessarily for every imaging session.

As the lamp ages or if you notice that there are unwanted non-uniformities in the images, you can take new reference images by choosing References in the drop-down menu. Taking new reference images can take 15—20 minutes, but it is not an especially challenging task. We recommend that your lab decide upon a procedure that specifies when new references should be taken.

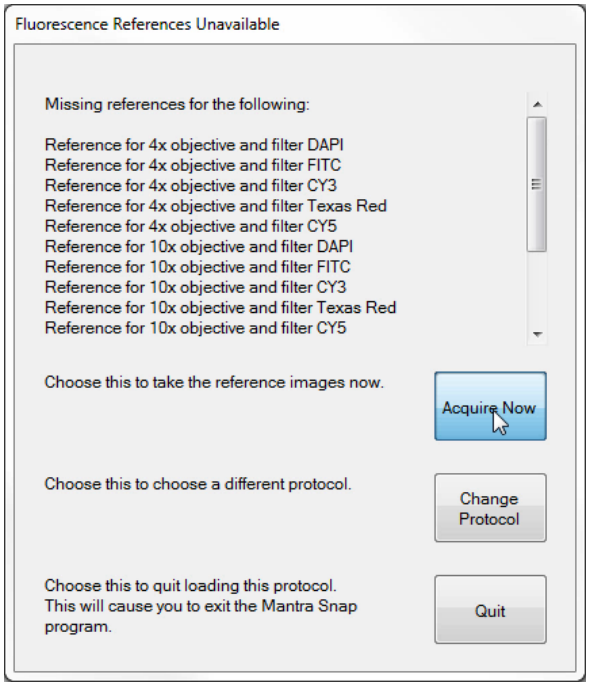

Fluorescence References Unavailable
Missing references for the following:
Reference for 4x objective and filter DAPI
Reference for 4x objective and filter FITC
Reference for 4x objective and filter CY3
Reference for 4x objective and filter Texas Red
Reference for 4x objective and filter CY5
Reference for 10x objective and filter DAPI
Reference for 10x objective and filter FITC
Reference for 10x objective and filter CY3
Reference for 10x objective and filter Texas Red
Reference for 10x objective and filter CY5
Choose this to take the reference images now.
Acquire Now
Choose this to choose a different protocol.
Change Protocol
Choose this to quit loading this protocol. This will cause you to exit the Mantra Snap program.
Quit

Figure 28. Taking reference images is made easy by following the directions in the Mantra Snap program. Good reference images ensure the high-quality images from your slides. You do not have to take reference images every time you use the Mantra system, but you certainly must take them the first time you use it.

If you have already taken references, you can view them using the references editor. References are stored for two trinocular positions; Camera Only and Shared. Adjust your trinocular to one of those two positions, then click **View References** to see the reference images for that position.

If you would like to retake your references, click **Take References**.

Click **Done** when you are finished, or use the menu to select another editor.

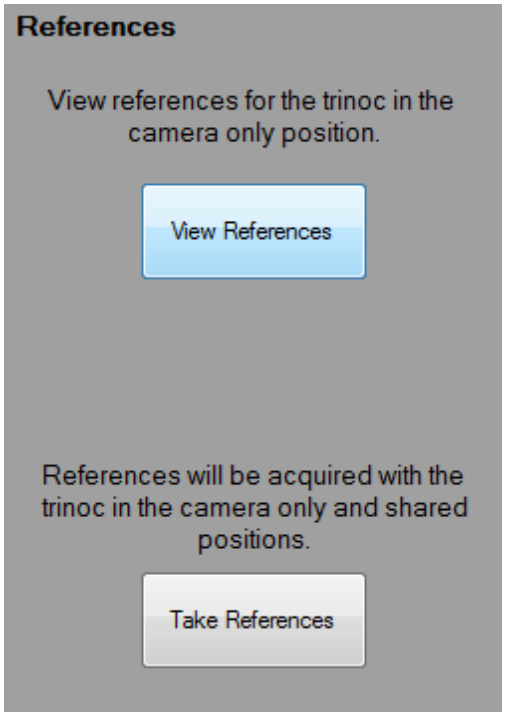

References
View references for the trinoc in the camera only position.
View References
References will be acquired with the trinoc in the camera only and shared positions.
Take References

Figure 29. Fluorescence References

Snap Rules

Select **Snap Rules** from the drop-down menu items under the Settings button. This setting area lets you select which fluorophores you are using and the order in which each fluorophore is imaged. Snap Rules do not appear when you are in brightfield mode.

You can click on the drop-down menu next to each number and choose a band.

Click **Add Band** to add an additional band or click on the red *X* to the right of an existing band to delete the band. You need at least one band to take pictures, and should only use a band once.

Images will be acquired in the order each band appears, top to bottom. So if you have a preferred order, add your bands in that order. You can also adjust the order afterwards by changing each selected band via the drop-down menu selector.

The following fluorescence bands are loaded at the factory:

- DAPI
- FITC or AF488
- Cy3 or TRITC
- Texas Red or AF594
- Cy5
- Cy7 (optional)

NOTE: the bands that appear in the drop-down menus have been previously defined in the Hardware Setup program (described in Appendix A 50). You cannot add bands that do not already appear in the Hardware Setup program.

PerkinElmer recommends that you have the **Use Saturation Protection** checkbox selected. This will only use auto-exposure when necessary, protecting you from unintentional overexposure and 'clipping' potentially valuable signal.

As a safeguard against inadvertently taking an image when the trinocular optical path switch is not in the desired position (e.g. no light or not enough light going to the camera), you can select among the following options in **'Prior to acquisition, the trinoc must be at':**

- Camera Only
- Shared
- Either Camera Only or Shared

Mantra Snap will warn you if the optical path switch is in a position that you do not desire. In order to ensure that your data is quantitative, your trinocular should always be set to the same position for acquisition.

Click **Done** when you are finished, or use the menu to select another editor.

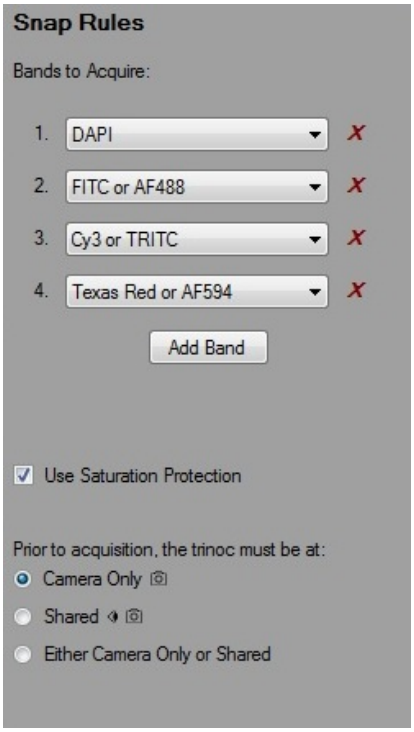


Figure 30. The Snap Rules editor allows you to select which fluorescence bands to acquire.

Exposures

Select the **Exposures** drop-down menu item from the Settings button to go to the Exposures settings area. Exposures will be displayed for your current objective.

If you have loaded a previously saved protocol, your saved exposures will be displayed. If you haven't assigned exposures for this objective and band, a default exposure is used.

Select the first Imaging Band (e.g. DAPI) and locate the brightest area on the sample before clicking **Autoexpose**. The system will automatically change to the correct epi-filter, the shutter will be opened if it was previously closed, and the system will calculate the optimal exposure time.

After autoexposure is complete, the shutter will be returned to its previous position.

You can also manually select an exposure time in milliseconds by typing directly into the box in the **Exposure column** to the right of each of your imaging bands. Exposure times must be between 1 and 2000 milliseconds.

If you'd like to set exposures for a different objective, use the Objective Rocker Switch on the hardware to select a new objective.

If you'd like to set exposures for a different imaging band, you can either select the Imaging Band or use the Epi-Filter rocker switch. The system will automatically highlight the current Imaging Band.

Set exposures for all of your imaging bands and for the objectives that you want to use.

Click **Done** when complete and you will be taken back to the Mantra Snap editor, or select another editor from the Settings menu.

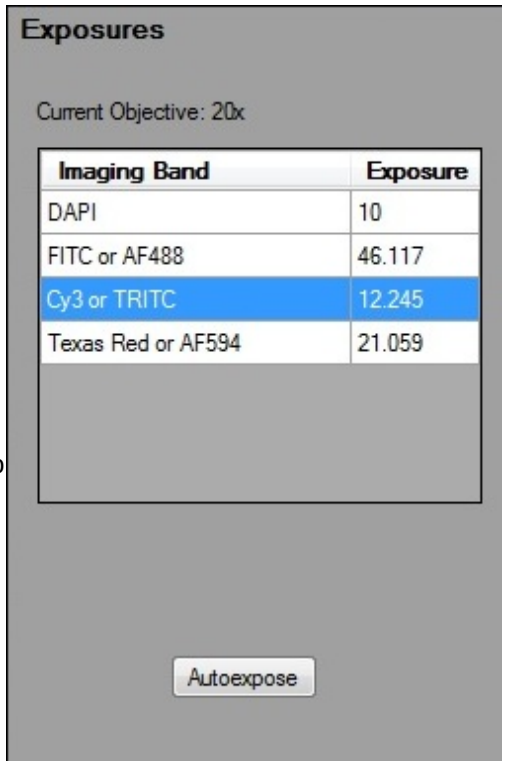


Figure 31. The Exposures editor lets you autoexpose each band for each objective or manually set the exposure time in milliseconds.

NOTE: If you would like to set exposures for bands that are not used for imaging, you can do so by selecting the correct filter using the Epi-Filter Rocker Switch on the hardware.

Use the Non-imaging band exposure field to autoexpose or manually set your exposure

Figure 32. Set exposures for non-imaging bands.

2.5.2 Taking a Fluorescence Image

Locating Your Sample

Locate a suitable fluorescence slide and place it onto the stage below the objectives. You may set the trinocular optical path switch so that some or all of the light goes to the eyepieces. Choose the objective and select an epi-fluorescence filter by clicking on the Epi-Filter and Objective switches located on the Mantra's lower front control panel.

Figure 33. You can use the Epi-Filter rocker switch to select the epi-fluorescence filter that you wish to use, and the Objective rocker switch to select the objective you want to use. In fluorescence mode, the blue light above the FL (fluorescence) label is lit. The BF Lamp knob for setting the brightfield illumination level is deactivated when you are in fluorescence mode.

With your sample on the stage, locate a portion of the slide that you wish to image. You may use the Stage Control knob, shown in Figure 34, to move the stage or handle the slide directly. The knob is normally located to the right of the stage, next to one of the large focusing knobs. You can have the stage control knob, as well as the main focusing knob moved to the opposite side. Contact PerkinElmer's Service and Support team[11] for details on performing the procedure.

Figure 34. Use the Stage Control knob located by default on the right side of the Mantra instrument to move the microscope stage along the X- and Y-axes.

Set the level of fluorescence illumination by adjusting the knob labeled Light Output on the front of the Mantra Fluorescence Illuminator to 25%. This should be sufficient for most fluorophores. If you find that the level is too low for sufficient signal from the emitted fluorescent light, turn up the level. However, if you find that your images are washed out (the peak levels are 'clipped'), turn down the level.

> *NOTE: PerkinElmer recommends setting the light output level from the Mantra Fluorescence Illuminator sufficient to allow your fluorphores to be visible in the eyepieces or on the monitor, being mindful that levels that are very high increase the chance that your tissue may become photobleached, where the emitted signal weakens over time with repeated or prolonged exposure to the excitation light. Excessively high illumination levels can also result in the fluorescence signals*

appearing to be washed out—the highlights being 'clipped' and lacking detail.

Set the trinocular optical path switch so that all or part of the light goes to the camera and lets you see a live streaming image of the slide on the monitor.

NOTE: If this is the first time you have opened Mantra Snap, you might not see a clear image because you may need to set much longer exposure times for each fluorophore in your sample in the Exposures editor.

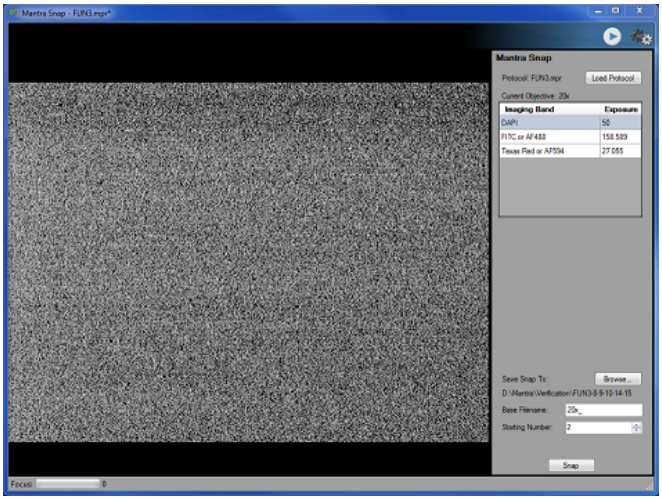


Figure 35. The Mantra Snap editor allows you to select a protocol, see your exposure times in milliseconds, and specify a location to save your images. You can also specify a base filename and a starting number for capturing sequences of images.

Destination Folder

Select a destination folder for your images by clicking on the Save Snap To: **Browse…** button at the bottom of the Mantra Snap Settings area to the right of the live image. The default location is D:\Data\MantraSnap.

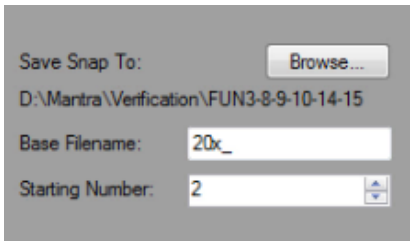


Figure 36. Mantra Snap Controls

NOTE: the Mantra computer comes with two hard disk drives, one for the Windows operating system and the Mantra Snap and inForm software (C: drive) and one for your data (D: drive). PerkinElmer recommends saving your data to the D: drive and making back-up copies whenever possible to avoid data loss in case your hard disk drive ever fails. Your institution will almost certainly have a network to allow access to larger storage areas for your data. The Mantra computer comes with a high-speed Gigabit Ethernet port for this purpose.

Base Filename

Enter a new name in the **Base Filename** text box and choose a **Starting Number**. New images will be placed into a folder named after the base filename that you have entered. Those images will be similarly named with the base filename and a number, beginning with the starting number you have entered. The default starting number is 1 (one). The file format suffix is PerkinElmer's .IM3. This file format preserves relevant information from the Mantra system including but not limited to the imaging mode, magnification, exposure times, stains that were present on the slide, and creation date.

So, if you entered a Base Filename of 'My IHC Slide' and chose a Starting Number of '5', the first image you take will be named 'My IHC Slide 5' (or 'My IHC Slide 5.im3' if you have chosen to have Windows

show file-type extensions) and placed into a folder named 'My IHC Slide'. The next image you take will be named 'My IHC Slide 6' and placed into the same folder. You may choose any starting number, but you will be warned if your choice inadvertently results in a file with the same name in the same location as an existing file. You will be given a choice to overwrite the existing file or to select a different filename.

Taking an Image

Unlike taking brightfield images using just one broadband (white) light source, fluorescence imaging uses one or more excitation wavelengths and corresponding epi-fluorescence filter cubes located in the epi-fluorescence filter turret between the trinocular and the objectives. Light passing through each epi-fluorescence filter cube (or the included quad-band filter cube) hits the specimen on the slide and results in emitted fluorescence light, which is rapidly captured by the solid-state LCTF and camera sensor.

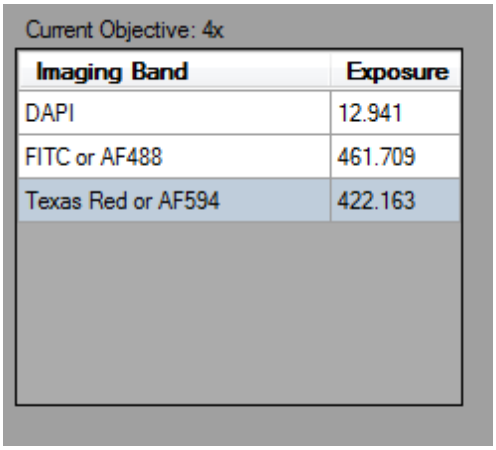


Figure 37. Mantra Snap Exposures

The Mantra Snap editor will show the current objective and highlight the current band and exposure time. If the current band is not an imaging band, it will be listed below the table.

> *IMPORTANT: Since many tissue specimens are subject to the phenomena called photobleaching, Mantra provides a shutter mechanism that can be activated by pressing the Shutter button on the handgrip on the left side of the Mantra instrument to protect the specimen from exposure to the excitation light when the slide is not actually being observed or imaged.*

Focus the image using the focus control knob on the Mantra microscope body. You can see the slide in real time on the monitor.

The green bar of the **Focus Indicator** in the lower left of the Mantra Snap program grows longer as the image displayed is sharper and becomes shorter as the image displayed is less sharp. The green bar may not extend to the full length of the indicator's space. So simply strive for the longest green bar and the highest number possible.

When you are ready, push the Snap button on the handgrip located to the left of the Mantra lower front control panel or click on the **Snap** button at the bottom right of the Mantra Snap program. You may observe the slide through the eyepieces or watch the LCD monitor while snapping your images. The system will acquire imagery of your imaging bands, returning back to the original epi-filter when it is done.

> *NOTE: If you hold down the Alt key on your keyboard while clicking on the Snap button in the Mantra Snap program, the button changes from Snap to Snap Color and you will simply save a color (TIFF) image instead of a full multispectral .IM3 file.*

The Mantra Snap program will warn you if you have inadvertently set the trinocular optical path switch directing all of the light to the eyepieces (and none to the camera) if you try to snap a picture when in that mode. You can take pictures with either some or all of the light directed to the camera.

You can click on the **Full-Screen Mode** button and the image will fill the Mantra Snap window, hiding the Settings Area. This can be very useful if you are only using the hardware controls and wish to have as large an image as possible on the screen.

Take a few more images to familiarize yourself with the Snap process. Next, you could set up a Post-Process procedure by going to the Settings button and choosing Post Processing. You can refer to the inForm User's Manual, version 2.1.0 or later, for additional details on the program's capabilities and features.

In order to save you from flipping back and forth amongst the two separate user's manuals for this particular exercise, the directions in Processing a Fluorescence Image in inForm 41 pertain to the inForm Advanced Image Analysis software program that comes with each Mantra system.

2.5.3 Processing a Fluorescence Image in inForm

Under normal circumstances, you would capture images from single-stained slides, as well as from some unstained tissue, in order to build up a 'library' of spectra (precise colors that are really unique multispectral profiles of your stains). PerkinElmer calls this a 'Stain Store' of the stains and fluorophores that you would see in the normal course of your research.

To help get you started, PerkinElmer includes some commonly used and characterized fluorescence spectra, referred to as 'synthetic' stains because they were not captured and characterized on your particular Mantra system. However, these synthetic stains will be close enough for instructional purposes that we will use them here and note when you could use single-stained samples if and when they are available to you. We highly recommend having an unstained slide in order to obtain the spectra of your tissue sample's autofluorescence.

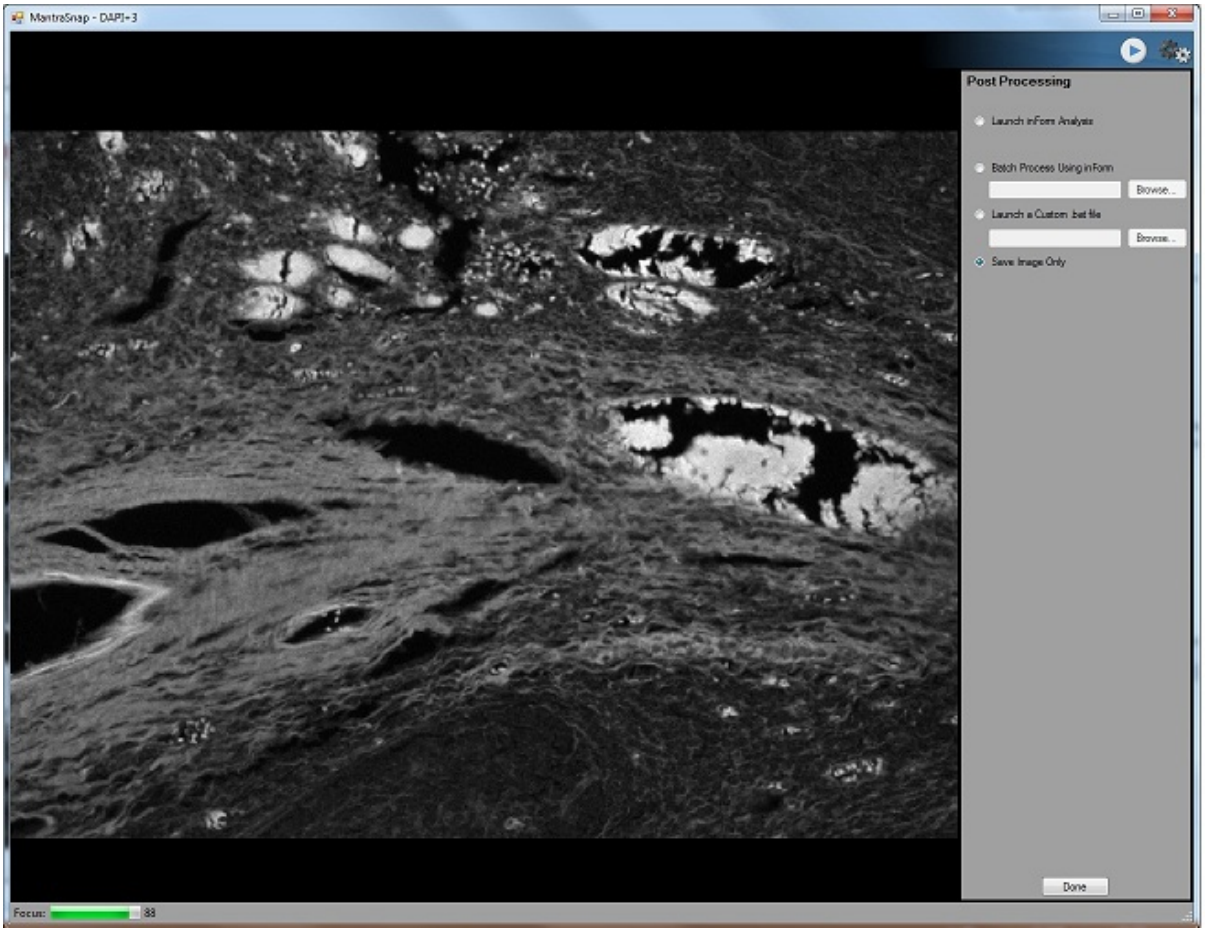


Figure 38. You can configure Mantra Snap to simply save your images, or you can configure it to automatically launch the inForm Advanced Image Analysis Software program, perform a batch process using inForm, or even launch a custom Windows .bat file.

In the Post Processing settings editor, seen in Figure 38, above, select **Launch inForm Analysis**. Go to the Mantra Snap editor, click Autoexpose or manually set your exposures in the Exposures editor, return to the Mantra Snap editor and **Snap** a picture of your stained sample slide. Then **Snap** a picture of your unstained slide. For purposes of instruction, we shall presume the slide contains several multiplexed stains: DAPI-counterstain, Cy3-cytokeratin, CD4-FITC, CD8-Alexa Fluor 594, CD20-Cy5. Shortly after the images have been acquired, the inForm program window will appear as shown in Figure 39, below.

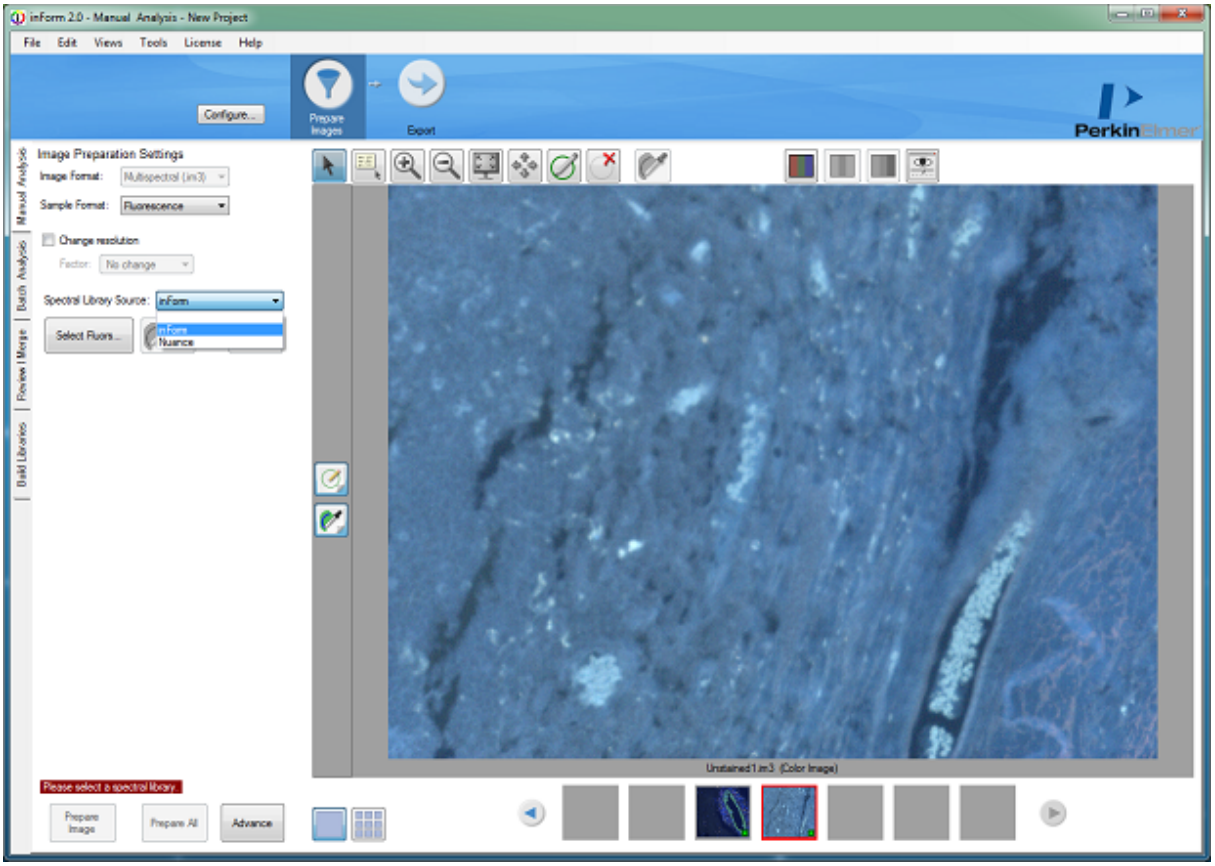


Figure 39. If you have chosen to open your image with inForm after Snapping a picture with Mantra Snap, the inForm program window will open with your image(s) in the display area.

The inForm program will automatically detect the type of image (.IM3, fluorescence). As shown in Figure 41, select **Spectral Library Source: inForm** from the drop-down menu in order to chose the spectra in your sample. This presumes that you do know the fluorophores present in your sample, of course.

Based on our example, choose the following synthetic stains

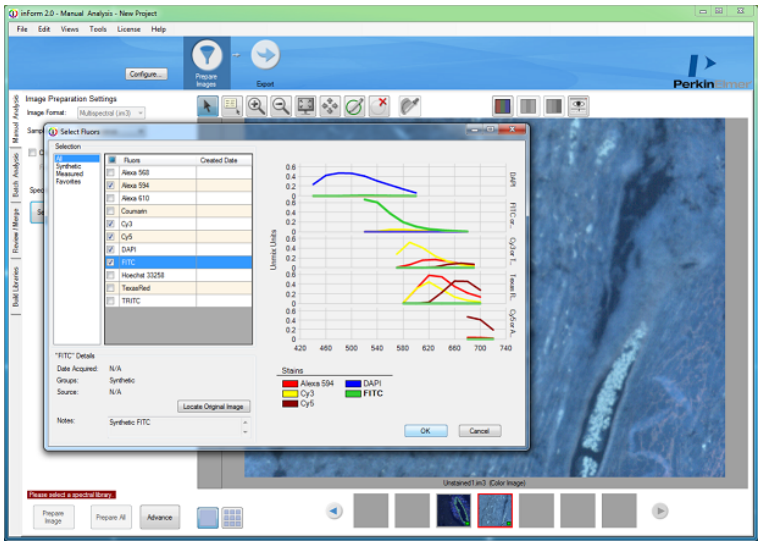


Figure 40. Select the fluorophores that are present in your sample from those in the Synthetic category in the Select Fluors window.

from the Select Fluors window that pops up: Alexa Fluor 594, Cy3, Cy5, DAPI, and FITC. Click OK.

You can enter markers to be associated with each fluorophore by clicking the **Edit Markers**... button. Simply enter the marker names into each text box and click OK.

Next, click on the **Select Autofluorescence** button. With the eyedropper pointer, draw in the unstained tissue to select the autofluorescence spectra and then re-select the multiplexed tissue image. Click the **Prepare Image** button.

The inForm program allows you to view your unmixed images in a variety of ways, including fluorescence images as if they were actually brightfield IHC images. You can even view each fluorophore as a color stain over a DAPI counterstain, as shown in Figure 41. Of course, you can view your images in black & white or color on a dark background. The autofluorescence is a separate color, dark gray in fluorescence and pink eosin when viewing your image as if it were brightfield IHC.

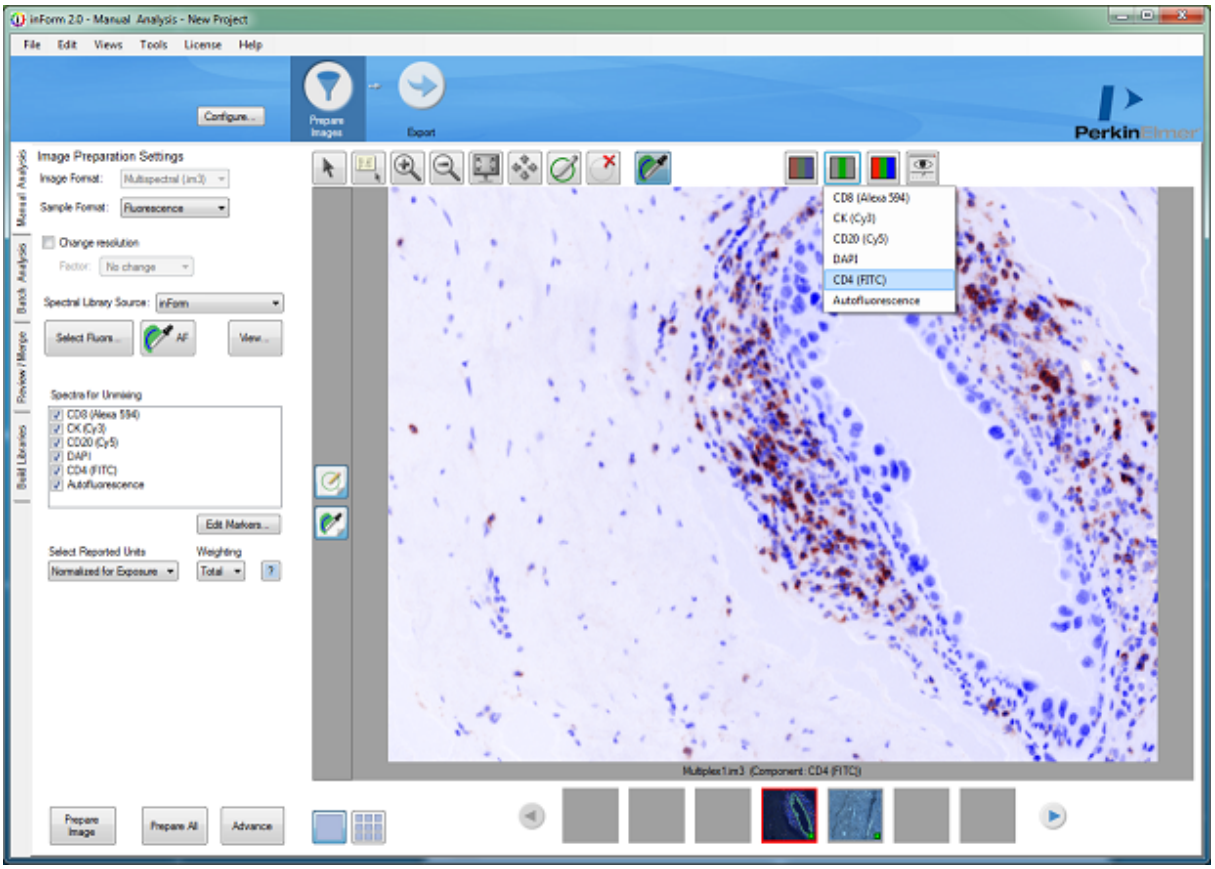


Figure 41. inForm can let you view your spectrally unmixed Mantra images as if they were brightfield IHC images. In this case, the FITC-CD4 is shown in brown overlaid onto a blue hematoxylin-like DAPI counterstain.

There are many other features in inForm, and you should refer to the inForm User's Manual for more detail.

Now that you have successfully chosen fluorophores and unmixed an image, you can return to the

Mantra Snap program and continue to take images. As long as the inForm program window is open, the new images will be processed just like the first image that you worked on. Of course, if you switch to a different slide that was prepared with different fluorophores, your results will not be consistent with the first slide.

3 Post Processing Your Images

By default, each time you snap a picture, the Mantra Snap program saves your image to the location that you selected for your Destination folder. But as demonstrated in the previous sections, Mantra Snap can do much more than that. You can tell the Mantra Snap program to automatically launch the included inForm advanced image analysis program and perform a pre-arranged operation, such as tissue and cell segmentation and phenotyping, on each of your images—even while you continue to snap pictures.

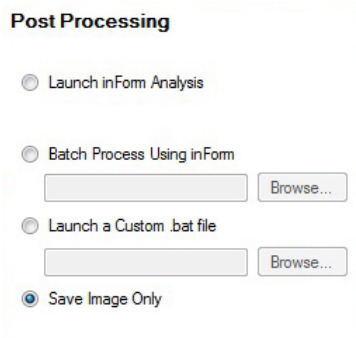


Figure 42. You have three options to post-process your Mantra images as well as the option to simply save them without any post-processing.

If this is the first time someone has opened the Post Processing editing area, the Save Image Only option will be selected. You can choose **Launch inForm Analysis**, which will start the inForm program (if it is not already running) after you snap a picture. If the program is open and an algorithm is loaded, inForm will automatically apply the algorithm to each new Mantra image as you take them. Figure 43 shows a phenotyping algorithm developed inForm that can be applied to new images are captured in Mantra.

You can choose **Batch Process Using inForm** which will not bring up the actual inForm user interface, but will run a specified algorithm (selected using the Browse… button) on each picture you snap in the background.

You can also choose **Launch a Custom .bat file** for additional expert control of automated processes within the Windows operating system environment.

Click **Done** when you've completed your selection, or use the Settings menu to navigate to another editor.

For more information on the capabilities of the inForm program, see the inForm User's Manual.

> *NOTE: This automatic transfer of Mantra Snap images to inForm is only available with inForm version 2.1 and later.*

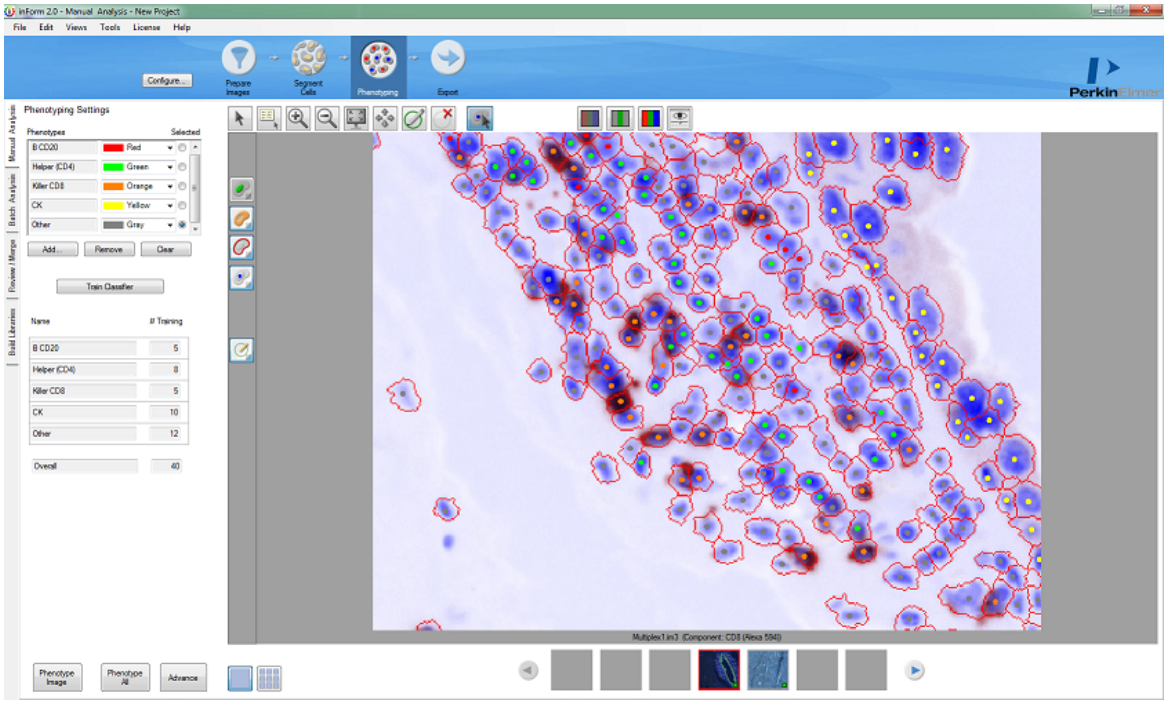


Figure 43. The PerkinElmer inForm Advanced Image Analysis software program shown here has automatically post-processed an image, phenotyping selected cells, taken with the Mantra Snap program. An image-processing algorithm had been created using a previously captured image and can be applied to subsequent images taken with a Mantra microscopy workstation in fluorescence mode.

4 Frequently Asked Questions and Troubleshooting

Why do I occasionally see portions of my sample turn a solid color such as red, green, or blue when I am rapidly moving the stage or switching objectives?

This is completely normal. In order to obtain such high spatial and spectral resolution with its scientific-grade CCD imaging sensor, the Mantra contains a solid-state liquid crystal tunable filter (LCTF). The LCTF rapidly changes wavelength (color) within milliseconds during live imaging, so that you see a high-quality color image on the screen.

Moving the stage rapidly or switching objectives can briefly cause just one color to be displayed while the filter is tuning from one color to another. When you snap an image, it actually cycles through many different wavelengths of light within a few seconds, taking a picture at each wavelength, and then the Mantra Snap program combines them into a multi-layered file containing all the information necessary.

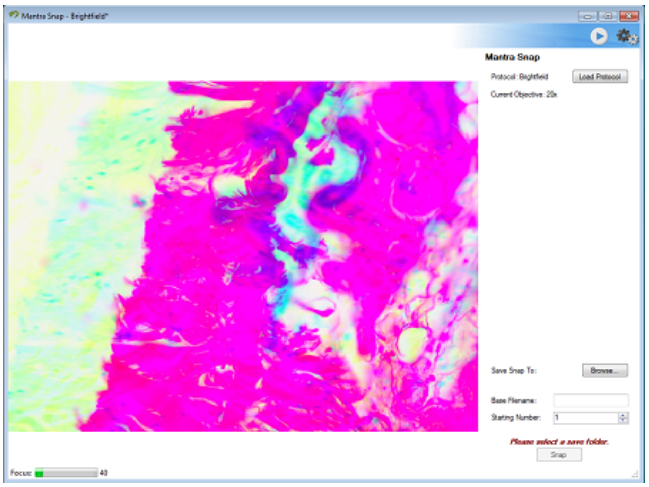


Figure 44. Rapidly moving the microscope slide stage can cause brief color shading as the specimen moves before the solid-state tunable imaging filter, which enables multispectral color acquisition, can complete a full cycle.

When I take pictures of slides that have been prepared using a near-infrared stain such as Alexa Fluor 750 or Cy7, is the Mantra system capturing images in the near-infrared part of the spectrum? I thought that the wavelength range of the tunable filter inside the camera module only tunes from 440 to 720 nm?

When taking pictures of near-infrared stains such as Cy7 or Alexa Fluor 750, the Mantra only needs to take an image at one wavelength, 770 nm. Unlike in the visible-wavelength range, there is little autofluorescence in the near-infrared wavelength range, and the acquisition of many wavelengths in order to spectrally separate the true signal from background is not necessary. In addition, near-infrared stains are distinct enough from visible-wavelength stains that there is no difficulty separating them.

When I am not using my Mantra system, should I switch it off?

Yes, in addition to saving electricity, switching off the Mantra will prevent overheating if the ambient temperature of your laboratory is relatively high or if you use a dust cover over the system. See the Mantra operating environment specifications 9. Keep in mind that the Fluorescence illuminator needs at least 30 minutes to arrive at its operating temperature and at least 30 minutes to cool down after you have finished using it.

WARNING: Do not place a cover over the Mantra instrument or the fluorescence illuminator while they are powered up or immediately after power down.

I am trying to extract a pure hematoxylin signal from a singly stained slide, but after I open the image that I took in Mantra in the Build Libraries tab in inForm, I don't see hematoxylin in the Stain drop-down menu. How can I add hematoxylin to the choices?

The Stain drop-down menu items in the Build Libraries tab are populated from the Stain Store, as described in the inForm User's Manual in Understanding the inForm Work Area, Stain Store Management and Build Libraries..

You might not have selected hematoxylin as a stain that your lab uses. Go to Tools >> Stain Store Manager and click on Choose Which Types of Stains and Fluors You Lab Uses.

Click on Brightfield under Family Name and then select the Hematoxylin checkbox on the right.

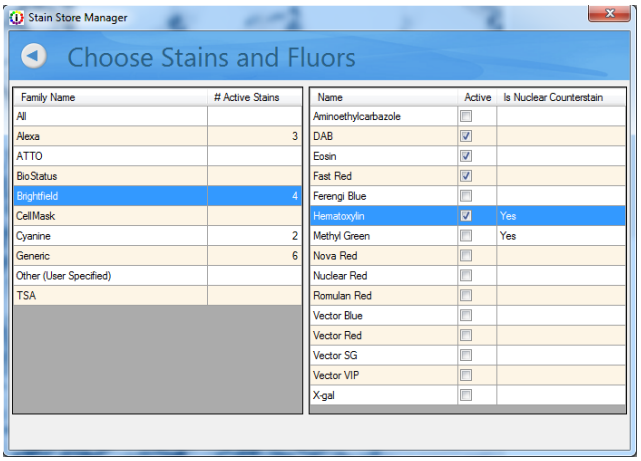


Figure 45. You can add stains and fluorophores to the Stain drop-down menu in the inForm Build Libraries tab.

Close the Stain Store Manager and return to the inForm Build Libraries tab. Hematoxylin will now appear in the drop-down menu.

Of course, you can also measure new stains and fluorophores that do not already exist in the inForm Stain Store. See the inForm User's Manual, Building Libraries for more details.

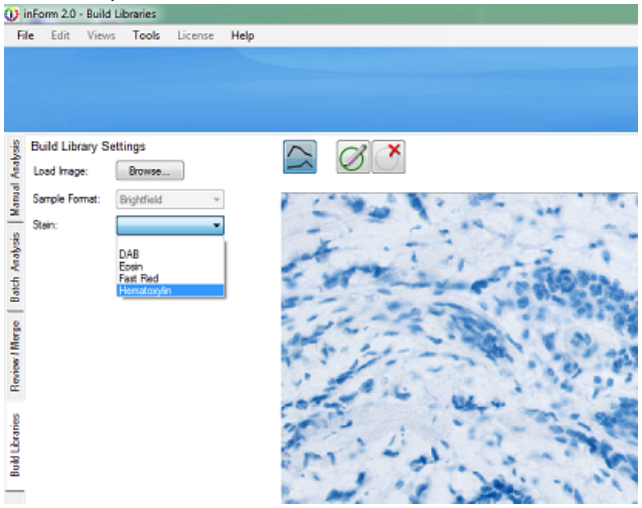


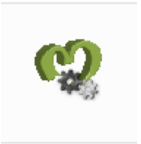
Figure 46. Once you have selected a stain or fluorophores in the Choose Which Types of Stains and Fluors You Lab Uses window of the Stain Store Manager, the stain or fluorophore will appear in the Stain drop-down menu in the inForm Build Libraries tab.

5 Appendix A: Mantra Hardware Setup

This appendix covers both the Mantra Hardware Setup program, as well as adjusting the Mantra microscopy hardware to suit your particular individual operating preferences (e.g. eyepiece viewing and ergonomic adjustments). Under normal circumstances you should not need to perform additional setup or maintenance procedures. If you have any questions, do not hesitate to contact PerkinElmer's Technical Support 11 team.

- Mantra Hardware Setup Program 50
- Mantra Microscopy Hardware Adjustment 58

5.1 Mantra Hardware Setup Program



When your Mantra system is first set up by the PerkinElmer technician who visited your laboratory, the Mantra Hardware Setup program is run, allowing the technician to configure your hardware with the proper objectives, epi-fluorescence filters, communications settings, as well as to properly align the optical system using the well-known Koehler method of alignment.

Unless you make changes to your hardware, you should not have to launch the setup program. You could periodically launch the program in order to perform Koehler alignment if you believe you're your condenser and field-stop settings might have been inadvertently changed. Information is provided here for reference purposes.

The Mantra Hardware Setup program can be launched from Programs\PerkinElmer \Mantra\1.0.0

Objectives

If you are instructed by PerkinElmer to add or remove an objective on your Mantra system, you can account for that change in the Objectives section of the setup program.

The Turret Size defaults to 7 and is not editable. The Mantra hardware features a seven-position turret and the software requires the presence of 7 positions. All positions need not be populated, though.

Use the Available Objectives box to create or edit entries for each of the objectives mounted in the objectives turret.

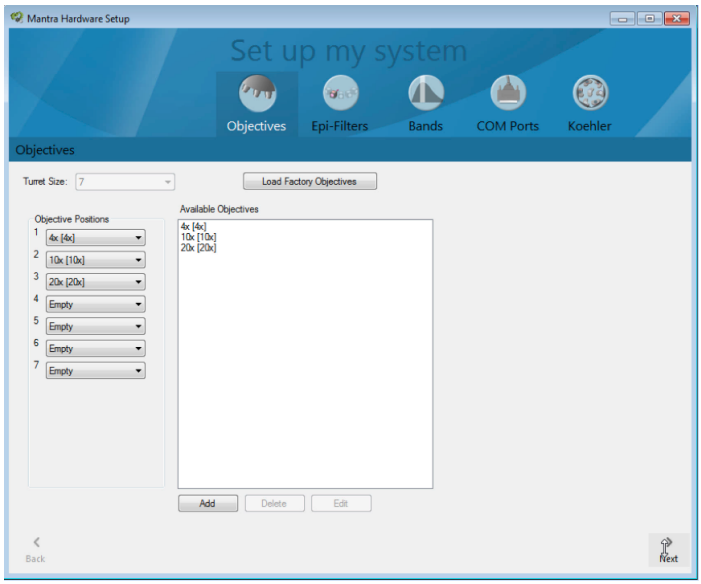


Figure 47. Adding or deleting objectives from within the Mantra Hardware Setup program.

- To add entries, click the Add button. Enter a short descriptive name and choose its nominal magnification. Click OK when finished. The new entry is added to the list.
- To edit the name of any objective, highlight the objective entry and click Edit. You can only edit the objective's name, not its nominal magnification. To change the magnification, delete the entry and create a new entry for the objective.
- To delete an objective from the system, first remove it from any objective position. Next, highlight the objective entry in the list and click Delete. Choose Yes to confirm you want to delete the objective from the system.

Use the Objective Positions boxes (1-7) to arrange the objective entries so they match the locations of actual objectives mounted in the turret. Note that as you assign objectives to their positions, the system prevents you from assigning the same objective to more than one turret position. To change an objective's location, remove it from the current location and then select the new location.

The Load Factory Objectives button automatically populates the Available Objectives box with the default factory objectives.

Click the Next button or the Epi-Filters button to continue with system setup. Or close the window if you are finished setting up or making changes to the hardware.

Epi-Filters

If you are instructed to add or remove an epi-fluorescence filter cube or cubes on your Mantra system, you can account for that change from the Epi-Filters section of the setup program

The Turret Size defaults to 8 and is not editable. The Mantra hardware features an eight-position turret and the software requires the presence of 8 positions. All positions need not be populated, though.

Use the Available Epi-Filters box to create or edit entries for each of the epi-fluorescence filters mounted in the filter turret. Notice that as you highlight each filter, the information for the filter displays in the space at the right.

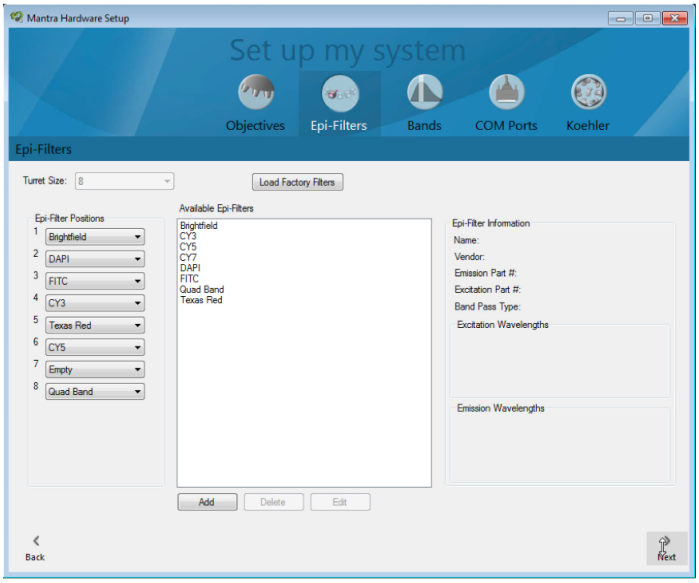


Figure 48. Adding or deleting epi-fluorescence filters from within the Mantra Hardware Setup program.

- **Add Filter:** To add entries for filters you have purchased separately, click the Add button. Then enter a short descriptive name. Enter the vendor and vendor part number. Select the Band Pass type, either Single-, Double-, Triple-, or Quad-band pass. Finally, enter the excitation and emission cut-on and cut-off wavelengths, in nanometers. Click OK when finished.
- **Band Name:** Whenever you add a new epi-filter, you will be prompted to enter a "band name" for the emission cut-on/cut-off range you entered. Every epi-fluorescence filter in the Mantra filter turret must have at least one band associated with it, and this is the filter's default band. Enter a name that includes the filter's specific target and intended wavelength range. See the next section, Setup System Bands, for more about bands.
- **Edit Filter:** The software will only let you edit filter names, and only the names of filters you create (not the default 'predefined' filters provided with the Mantra system). Highlight the filter entry and click Edit. While you can select a predefined filter and click the Edit button to view its parameters, you cannot edit them. You are prevented from editing the parameters of existing filters because they may have already been used in one or more experiments. If you were to edit these filters, the new settings would no longer agree with the settings that were in effect during the experiment. If you discover an error in the information of a recently added filter, you must add the filter again and then delete the filter with the error. Note that deleted filters are permanently deleted and cannot be recovered.
- **Delete Filter:** To delete a filter from the system, first un-mount the filter from its epi-filter position. Then highlight the filter entry in the list and click Delete. You can only delete filters you have created; you cannot delete any of the predefined filters. Choose Yes to confirm you want to delete the filter from the system.

Use the Epi-Filter Positions boxes (1-8) to arrange the epi-filter entries so that they match the locations of actual epi-fluorescence filters mounted in the turret, which are as follows:

- Position 1 – BF, brightfield (no filter cube present)
- Position 2 – DAPI

- Position 3 – FITC
- Position 4 – CY3
- Position 5 – TXRED, Texas Red
- Position 6 – CY5
- Position 7 – CY7, if present (optional)
- Position 8 – QUAD, quad-band filter for visualization through the eyepieces (optional)

The Brightfield filter is permanently assigned to position one; its position cannot be changed. Also, note that as you assign filters to their turret positions, the system prevents you from assigning the same filter to more than one position. To change a filter's location, first you need to remove it from its current position, and then select its new position.

The Load Factory Filters button automatically populates the Available Epi-Filters box with the default factory filters.

Click the Next or Bands button to continue with system setup. Or close the window if you are finished setting up the hardware.

Bands

You can also setup the bands associated with each epi-fluorescence filter.

The Bands panel mirrors the System Epi-Filters panel in that it lists all the installed epi-fluorescence filters. It also lists all of the Bands created for each of those filters. Bands specify how Mantra measures light over the filter's range of wavelengths. By adding multiple bands to a filter, you can quickly select how you want to use the filter during a specific protocol (e.g., within a narrow wavelength range, in high definition – 10 nm step size – mode, etc.).

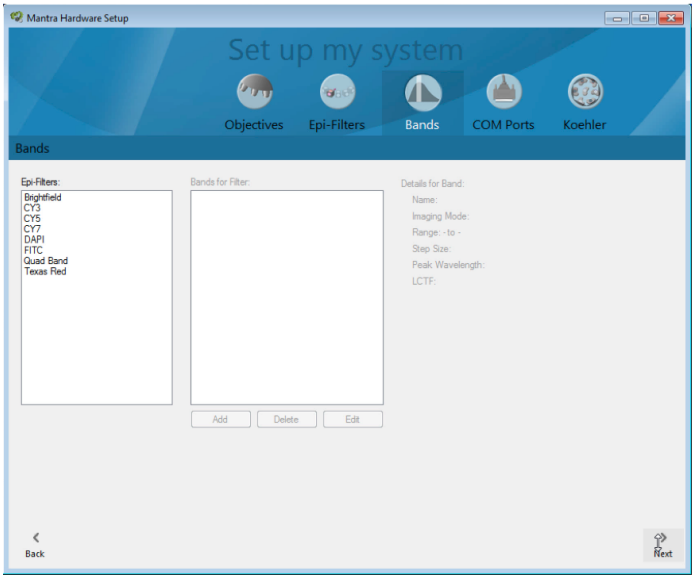


Figure 49. You can add or delete bands associated with each epi-fluorescence filter within the Mantra Hardware Setup program.

Clicking on an Epi-Filter reveals the bands that have been created for it. When you click on a band, the details of the band appear in the space on the right:

- **Name:** The name given to the band when it was created. Factory band names are not editable; you can only edit the names of bands you create.
- **Imaging Mode:** Always Brightfield for any bands created for the brightfield filter, and is always Fluorescence for bands created for any of the fluorescence filters.
- **Range:** The starting and ending wavelengths specified when the band was created. This range is limited by the emission filter. You can edit these values for non-factory bands only by clicking the Edit button.
- **Step Size:** The default step size is Normal (20nm). A High-Definition (10nm) step size is also available. You can choose either step size when adding or editing a band. Note that High-Definition bands will increase the file size significantly, and may not improve your data quality.
- **Peak Wavelength:** The peak wavelength is the wavelength that you will see live on the monitor and that will be used for focusing. Typically, the peak emission is approximately 20nm after the filter's emission cut-on wavelength, so this peak wavelength is used for the live view as well as for focusing. So, for example, if the epi-filter's emission cut-on wavelength was 633nm (or any value between 630–640nm), the default band's starting wavelength would be 630nm, and its peak wavelength would be 650nm. For any subsequent bands you create, the peak wavelength is 20nm above the band's starting wavelength.
- **LCTF:** The Mantra system always uses a broad bandwidth Liquid Crystal Tunable Filter, so this entry is display only, and cannot be changed.

COM Ports

If instructed, you can change the communications ports used by the Mantra system.

Use this panel to select which COM port connects to each device. Once a COM port is assigned to a device, it is unavailable for selection for the other devices. To reassign a COM port, remove it from the current device and then assign it to the new device.

The RS232 port provides communication to the microscope hardware and the USB port provides communication to the PerkinElmer electronics controller. Clicking on the **Validate COM Ports** button tests the selected ports to confirm proper operation (e.g. each COM port is controlling the proper hardware, so that you do not receive error messages upon Mantra Snap software program startup.).

NOTE: Under most circumstances, the RS232 port will be assigned COM1.

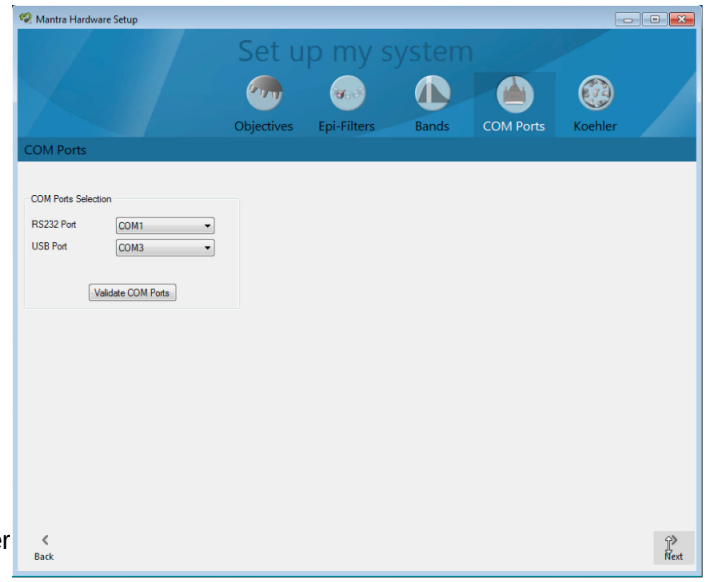


Figure 50. The communications setting can be adjusted from within the Mantra Hardware Setup program.

Koehler Alignment

Finally, you can follow step-by-step instructions to properly align the Mantra optical imaging system using the well-known Koehler alignment method. Follow the steps as they appear when you click Next. You can always go back if you are unsure if you correctly followed the instructions.

IMPORTANT: If you perform Koehler alignment, you should re-take your brightfield references.

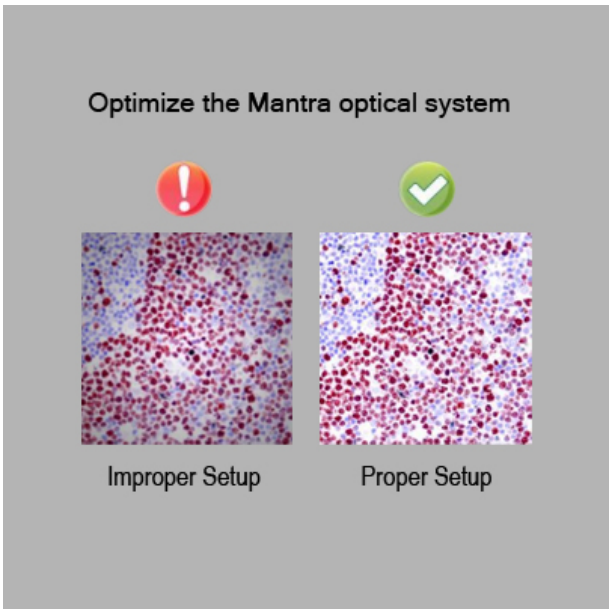


Figure 51. The Koehler alignment technique can ensure optimum quality of your images with good uniformity across the image, excellent contrast, and maximum efficiency of your light source.

Step 1. Select a brightfield slide with good detail that you can focus on. Place it on the microscope stage and focus.

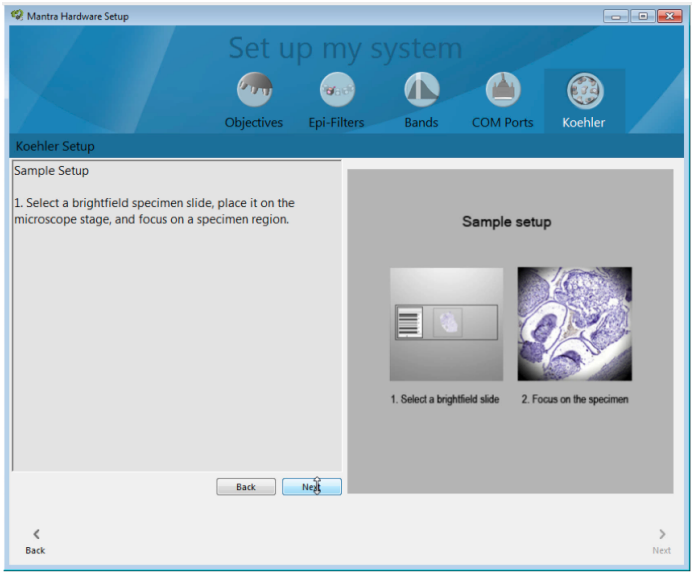


Figure 52. Step 1 of the Koehler alignment procedure requires a brightfield slide with good detail that you can properly focus upon.

Step 2. Use the Objective rocker switch on your Mantra system to select the highest magnification (20X or 40X, depending on your configuration). Close down the field stop by rotating the ring located around the brightfield illuminator (highlighted in yellow in Figure 53). Then adjust the height of the condenser by turning one or both of the condenser height adjustment knobs (highlighted in red in Figure 53) until the edges of the field stop are sharp. Finally, use the Objective rocker switch to select your lowest magnification (4X) and open the field stop until your field of view is unobstructed.

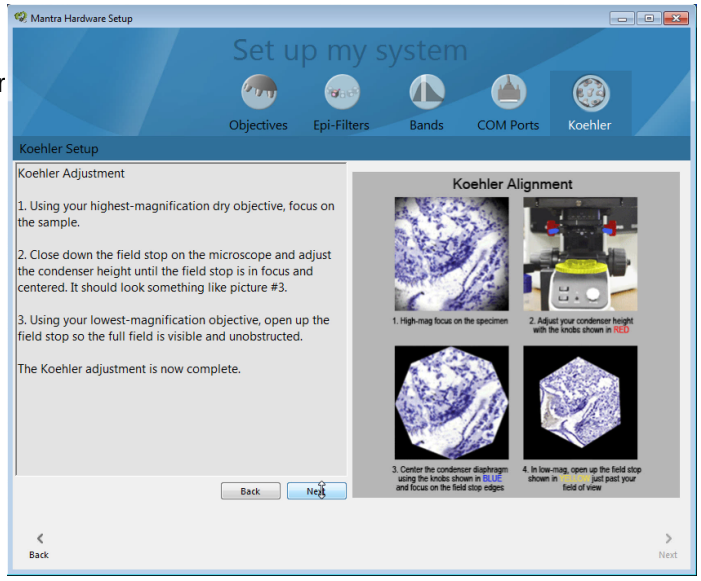

Mantra Hardware Setup
Set up my system
Objectives
Epi-Filters
Bands
COM Ports
Koehler
Koehler Setup
Koehler Adjustment
1. Using your highest-magnification dry objective, focus on the sample.
2. Close down the field stop on the microscope and adjust the condenser height until the field stop is in focus and centered. It should look something like picture #3.
3. Using your lowest-magnification objective, open up the field stop so the full field is visible and unobstructed.
The Koehler adjustment is now complete.
Back
Next
Koehler Alignment
1. High-mag focus on the specimen
2. Adjust your condenser height with the knobs shown in RED
3. Center the condenser diaphragm using the knobs shown in BLUE and focus on the field stop edges
4. In low-mag, open up the field stop shown in GREEN just past your field of view
Back
Next

Figure 53. Step 2 of the Koehler alignment procedure requires you to center and focus on the edges of the field stop.

Step 3. Set the condenser numerical aperture (NA) to your preferred value (highlighted in green in Figure54). If you do not have a preference, a list is provided of the NA values for your objectives. PerkinElmer recommends first trying the highest value in the list.

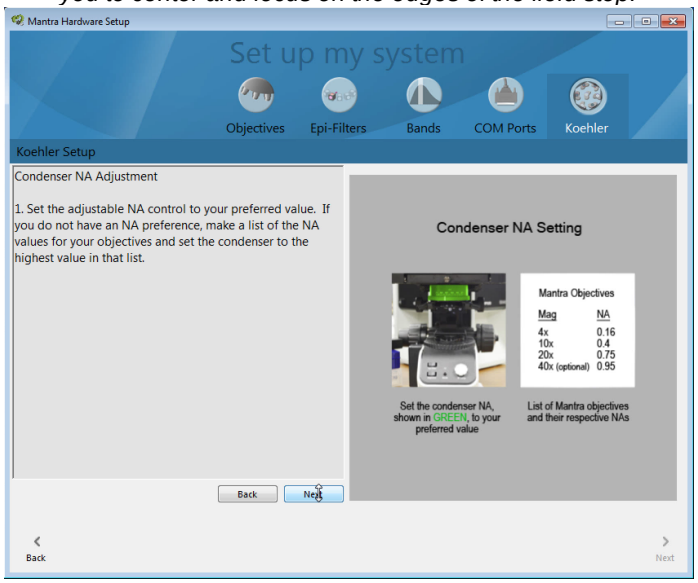

Mantra Hardware Setup
Set up my system
Objectives
Epi-Filters
Bands
COM Ports
Koehler
Koehler Setup
Condenser NA Adjustment
1. Set the adjustable NA control to your preferred value. If you do not have an NA preference, make a list of the NA values for your objectives and set the condenser to the highest value in that list.
Back
Next
Condenser NA Setting
Mantra Objectives
Mag NA
4x 0.16
10x 0.4
20x 0.75
40x (optional) 0.95
Set the condenser NA, shown in GREEN, to your preferred value
List of Mantra objectives and their respective NAs
Back
Next

Figure 54. Step 3 of the Koehler alignment procedure requires you select an optimal condenser NA setting.

Step 4. When complete, close the Mantra Hardware Setup program.

NOTE: PerkinElmer strongly recommends that you retake your brightfield references after completing the Koehler adjustment.

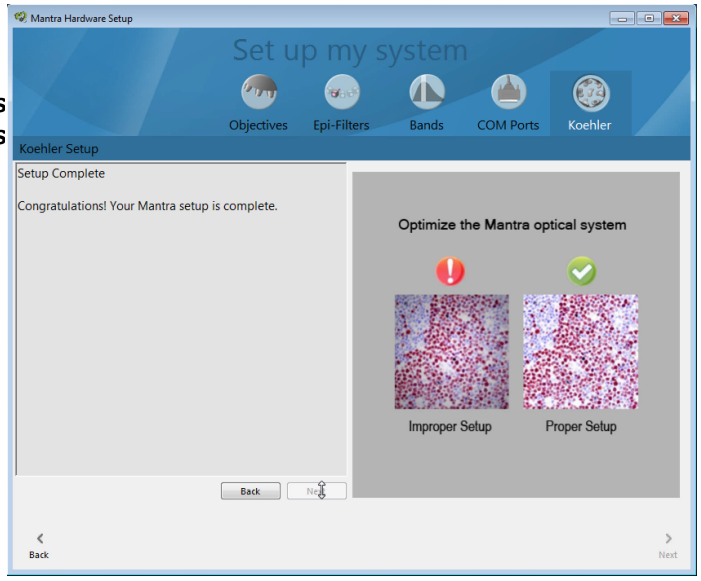


Figure 55. Step 4. When you complete the Koehler alignment procedure, you should see an improvement in image quality as compared with a poorly aligned system.

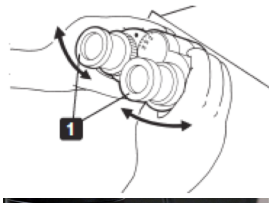
5.2 Mantra Microscopy Hardware Adjustment

The Mantra camera adapter is adjusted by the PerkinElmer technician during installation to maintain proper focus on the specimen when the image is displayed on the screen as well as when you are looking through the eyepieces, even with different objectives. This means that your setup is 'parfocal', although there may be some small variation. If you notice that the focus is significantly different, contact PerkinElmer Technical Support 11.

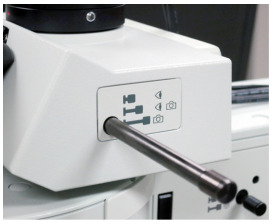
In order to optimize your comfort while using the Mantra instrument hardware, there are adjustments available to suit your own needs and preferences while using the microscopy controls. Each user can make adjustments to the following settings without negatively affecting the actual functionality of the Mantra workstation itself.

Mantra Eyepieces

Step 1.

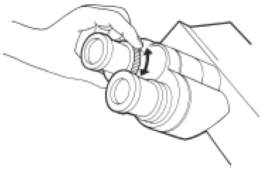


While looking through the eyepieces with 50% or 100% of the light sent to the eyepieces, adjust the interpupillary distance between the two eyepieces until you achieve binocular vision with both your left and right fields of view coinciding completely.



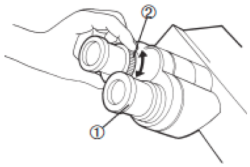
The Trinocular Optical Path Switch can be pulled or pushed into the trinocular in order to send the light to the eyepieces or both the eyepieces and the camera.

Step 2.



Set the objective to 20X (or 40X of your Mantra is equipped with a 40X objective) with 50% or 100% of the light sent to the Mantra camera. Focus on a detailed specimen on a slide on the slide stage. Then with 50% or 100% of the light sent to the eyepieces, adjust the diopter settings in the eyepieces to the image as seen through the eyepieces is equally sharp.

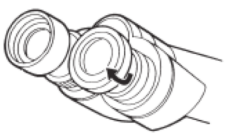
Normally the image on the computer monitor will be as sharp as the image seen through the eyepieces, but if it is not, and you prefer to fine-tune your eyepieces and then make adjustments to the focus while looking at the monitor before taking pictures, perform the following routine:



1. Set the diopter adjustment rings on both sides to scale ‘0’.
2. Engage a high-power objective (20X or 40X) in the light path, look into the right eyepiece with your right eye, and rotate the coarse and fine adjustment knobs to bring the specimen into focus. Do not use an oil-immersion objective.
3. Engage a low-power objective (10X or so) in the light path, rotate the right diopter adjustment ring to bring the specimen into focus. Do not touch the coarse and fine adjustment knobs during the focusing.
4. Looking into the left eyepiece with your left eye, rotate the left diopter adjustment ring to bring the specimen in focus. The above procedure adjusts the diopter with reference to the right eye, but it is also possible to adjust with reference to the left eye. In this case, read the above procedure by inverting ‘right’ and ‘left.’

If an eyepiece equipped with a diopter adjustment ring is inserted into the side equipped with another diopter adjustment ring of the observation tube, perform the above procedure without moving the diopter adjustment ring of the observation tube from the ‘0’ position.

Step 3.



The Mantra eyepieces are equipped with rubber eyeshades that can be folded down for users with eyeglasses or folded up to prevent extraneous light from entering the eyepieces and eyes.

Mantra Manual Controls

The left side of the Mantra instrument features a small lever located next to the tension adjustment ring that adjusts the amount of force necessary to rotate the larger coarse adjustment ring on the focusing knob. The lever is used to set the upper limit of the stage position to prevent inadvertent contact by the slide and objective. The center of the knob is used for fine focus adjustments.

Also on the left side are the Snap and Shutter buttons located on a handgrip attached to the side of the Mantra microscope body, shown in Figure 56.

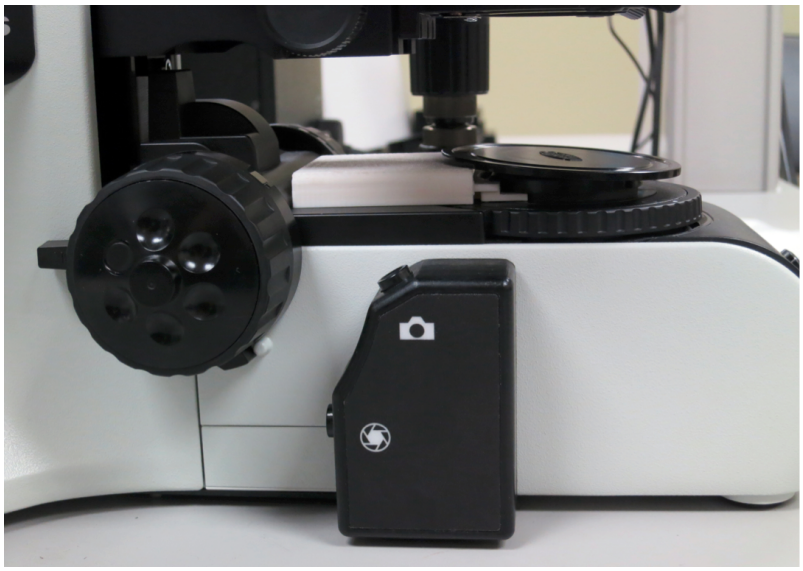


Figure 56. The left side of the Mantra instrument shown here with the knob for focus adjustment and the small lever (behind the knob) to set the upper limit of the stage position on the left. To the right of the knob is the handgrip with the Snap button on top and the Shutter button on the side.

The right side of the Mantra instrument features additional coarse and fine adjustment rings around a focus knob, as well as the vertically oriented X (bottom) and Y (top) stage control knobs, shown in Figure 57.

Under normal circumstances you should not have to adjust the height of the microscope stage. If the stage height is inadvertently changed, contact PerkinElmer to obtain instructions on setting the stage to the proper height.

Similarly the condenser knobs and rings should not have to be adjusted after initial setup. However, if they are inadvertently changed, you can follow the instructions in the Mantra Hardware Setup Program to perform a Koehler alignment that will help you make the correct adjustments for optimum imaging quality.

Figure 57. The right side of the Mantra instrument shown here with the knob for focus adjustment on the right. To the left of the knob are the stage controller knobs for X and Y axis control of the specimen under the objectives.

6 Appendix B: Installing the Mantra Software

The Mantra software suite, which includes Mantra Snap and two seats of inForm advanced image analysis software's Tissue Finder, is pre-installed and activated at the factory.

If directed to do so, you can perform a software installation using software installer for Mantra Suite of software, that includes Mantra Snap and inForm. The installer program will instruct you to unplug the USB connection on the Mantra interface panel if it is not already unplugged. This is very important in order for the installation to proceed correctly.

IMPORTANT:

Prior to running the installer program, unplug the Mantra USB connection.

Once installed, the Mantra Snap software will not run without a working Mantra Instrument attached.

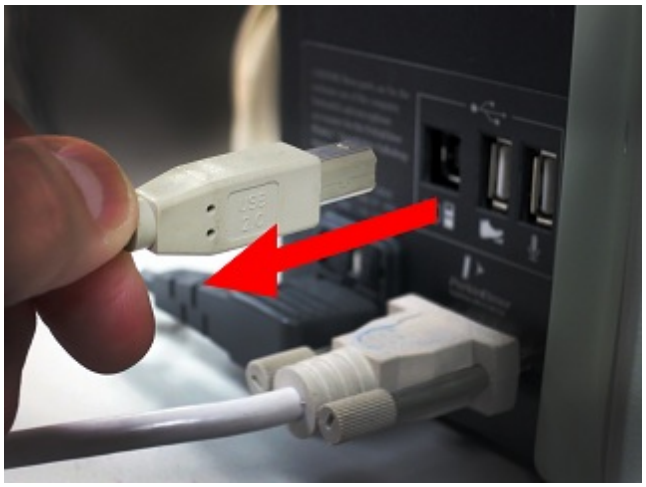


Figure 58. You must unplug the MantraUSB connection on the Mantra interface panel before performing an installation or an upgrade of the Mantra software.

You will need to be logged in as an Administrator on your Windows computer. To activate your Mantra Snap license online, start up the licensing app at

Start >> PerkinElmer >> Mantra >> 1.0.0 >> Mantra License App.

Have your 20 character license number ready and follow the instructions.

You can also reinstall inForm if necessary. Please see the section "Activating the License" of the inForm Manual.

Mantra License Application
Mantra License
Please have your 20 digit license number ready before licensing.
Show License Info
Activate License

Figure 59. Mantra Licensing Application

7 Appendix C: Mantra Fluorescence Illuminator: Setup and Maintenance

The Mantra Fluorescence Illuminator is a 200W module that provides epi-fluorescence illumination of slides on the Mantra microscope stage. It has 6 levels of illumination (0%, 10%, 25%, 50%, 75%, and 100%) using a self-aligning, temperature-controlled 200W metal halide bulb.

The Mantra Fluorescence Illuminator is normally unpacked and installed by a PerkinElmer-trained technician. Unless specifically asked to perform the unpacking, installation, or setup, please contact Technical Support 11.

Setup Procedure

Ensure that there is a minimum of 100 mm (4 inches) clearance around the Mantra Fluorescence Illuminator at all times. Equipment and objects should be placed in such a manner that the power switch and disconnecting devices are accessible at all times.

Figure 60. The Mantra Fluorescence Illuminator should be placed in a location where there is at least 100 mm around it at all times.

Installing or Replacing the Bulb

Required Equipment:

- Hex Key (supplied with the unit)
- 200 Watt Metal Halide Bulb (P/N 132364)

WARNINGS:

- Use only the specified 200W Metal Halide bulb. Attempting to install any other bulb may cause damage to the unit.
- Do not touch the inside of the reflector of the bulb.
- The bulb is delicate, so handle it carefully.

1. Make sure the Illuminator is turned OFF and disconnect the power supply. (Wait 30 minutes to allow the bulb to cool after turning the unit off.)
2. Lay the Illuminator upside down on a flat, padded surface.
3. Remove the four hex screws shown below and remove the panel.
4. Carefully remove the bulb from the packaging:

   Do not touch the silver surface of the lamp or the PCB on the back of the bulb.

   Open the top of the carton, remove the V-shaped cardboard holder, push back the cardboard flaps, and lift the bulb out of the carton.
5. Turn the bulb so that the cables and connector hang down into the opened bulb housing.
6. Plug the brown connector from the bulb into the power socket in the bulb housing. Make sure it is pushed firmly into position.
7. Make sure the bulb is oriented so that the (Hg) label faces upwards. Then place the bottom side of the bulb into the groove in the bottom of the lamp housing.
8. Lift the spring towards the bulb. This pushes the bulb into the correct position. Click the spring all the way into the Lamp Spring Restraints to lock the bulb in place.
9. Slightly rotate the lamp in its holder and set it in the middle of its travel.

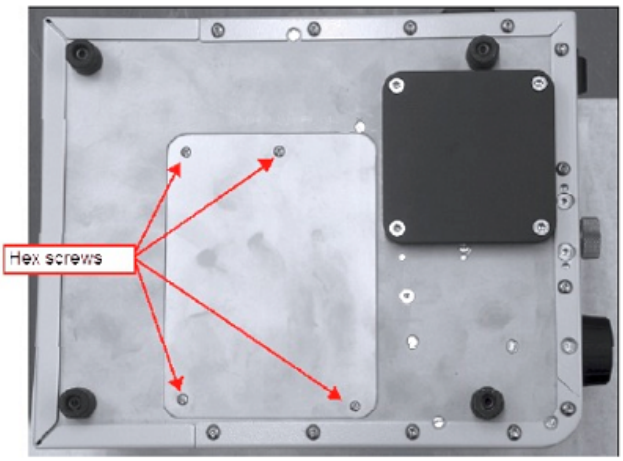


Figure 61. Mantra Fluorescence Illuminator bulb housing with the location of the four (4) retaining screws shown.

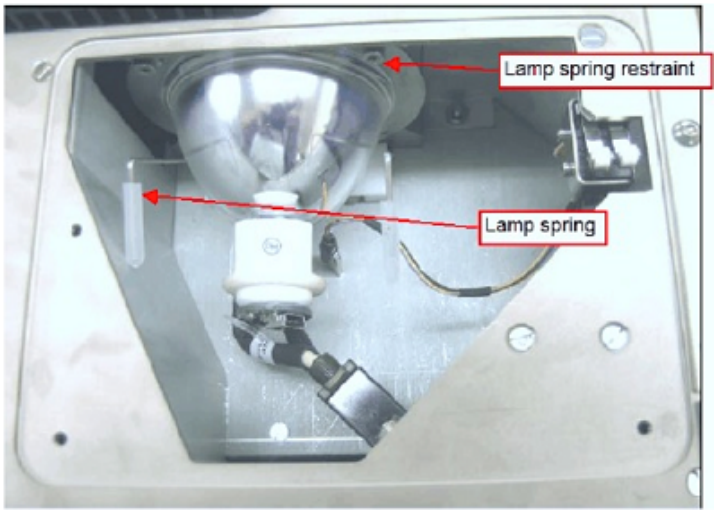


Figure 62. The 200W metal-halide bulb installed in the enclosure.

10. Plug the data connector into the base of the bulb.
11. Re-install the bulb housing cover using the four hex screws.

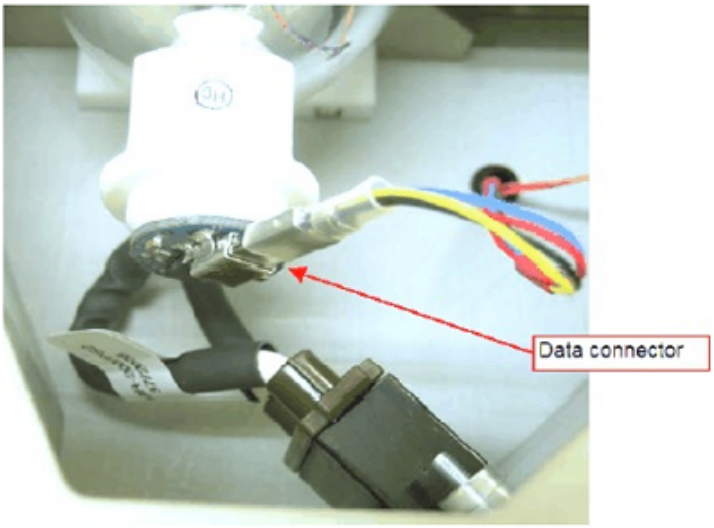


Figure 63. The 200W metal-halide bulb data connection.

Liquid Light Guide connection to the Illuminator and the Mantra instrument

Before turning the illuminator on, confirm that the Liquid Light Guide is properly and firmly attached to the front of the illuminator and the rear of the Mantra instrument. If it is not, follow the instructions below.

1. Position the illuminator on the bench so that none of the fan vents are obstructed.
2. Unpack the liquid light guide from the foil packaging and remove both plastic caps from the light guide.
3. Unscrew the connector on the front of the illuminator and fully insert the light guide. (Push the light guide in until it stops.)
4. Tighten the connector until tight to fully secure the light guide to the illuminator.
5. Loosen the screw on the back of the collimating lens and push the light guide firmly into the hole. Make sure it reaches the end stop, and then tighten the screw.
6. Attach the collimator to the Microscope's fluorescence illumination port.
7. Switch the illuminator on and adjust the collimator for light evenness:

   a. Loosen the silver lock ring.

   b. Unscrew the end of the collimator.

   c. When light is even, screw the silver lock ring forward, locking the collimator end into place.

NOTE: The liquid light guide has a limited lifetime, regardless of whether it is in storage or in use. However, the lifetime may vary, depending on climate conditions. Cold and humid environments tend to extend lifetime; hot and/or dry environments tend to shorten it. Even though UV performance does not markedly degrade during usage, it is recommended that the light guide be replaced before its expected lifetime expires. The formation of bubbles in the liquid degrade performance, and optical output may drop very rapidly.

Liquid light guides have an expected lifetime of 4 years. The suggested replacement interval is every 3 years. These figures are based on a temperature of 73.4 °F (23 °C) and 60% humidity.

If the system is used at ambient temperatures beyond the recommended range, it is likely that bubbles

could form inside the liquid. These bubbles may be reabsorbed by the liquid by storing the light guide at the recommended temperature for several days.

Starting the Mantra Fluorescence Illuminator

WARNING: Make sure that the power switch is in the OFF position when the unit is plugged in. Plugging the unit into a power outlet when the switch is in the ON position can result in damage to the unit.

Do not power up the fluorescence illuminator without the light guide attached to both the illuminator and the Mantra instrument.

Only power up the illuminator when it is on a flat, stable surface.

Confirm that the power switch is in the OFF position. Plug in the power cable to a power outlet. Switch the power switch to the ON position.

Although the illuminator will reach 70% output in 1 - 5 minutes, allow 30 minutes for it to reach operational temperature.

Shutting the Illuminator Off

WARNING: Do not switch the unit off within 5 minutes of switching it on. Not complying with this warning may result in damage to the bulb.

After switching the unit off, allow the bulb to cool for at least 30 minutes before changing the bulb or switching the unit back on. Failure to do so is likely to result in damage to the bulb.

When to Change the Bulb

The bulb is installed with a timer chip that counts the hours the bulb has been lit. Once the bulb reaches the recommended lifetime of 2000 hours, an audible reminder sounds each time the illuminator is switched on. See Installing or Replacing the Bulb 62.

- It is recommended that the bulb be changed at this point.
- To silence the alarm, press the Alarm Reset button located at the left of the display panel on the front of the illuminator.

Routine Maintenance

The internal dust filters require cleaning every 12 months of use.

1. Make sure the illuminator is turned OFF and disconnect the power supply. (Wait 30 minutes to allow the bulb to cool after turning the unit off.)
2. Remove the three screws that fasten the rear cover.
3. Lift the rear cover off.

4. Remove the filters.

5. Wash the filters in warm soapy water.

6. Rinse the filters in water and allow them to dry.

7. Reinstall the filters.

8. Reinstall the rear cover ensuring the isolator switches are beneath the cover.

9. Replace and tighten the screws.

If you need to clean the exterior surfaces of the illuminator, switch the power switch Off and unplug the power cable before wiping the exterior surfaces with isopropyl alcohol.

Alarms, Warnings and Actions

The table below lists the warnings that may appear on the display on the front of the Mantra Fluorescence Illuminator and the recommended actions to address the problem.

Message	Alarm Reason	Quiet Alarm	Action(s)
Bulb Fault 1	Software not recognizing hour count from bulb	No	Switch off unit. Check bulb for damage and check connections.
Bulb Fault 2	Bulb failure	Yes Hold Alarm Reset button for 5 - 10 seconds. Reset to clear alarm	Switch off unit. Check bulb for damage and check connections.
Over Temp Fault	Bulb over temperature	Yes Hold Alarm Reset button for 5 - 10 seconds. Reset to clear alarm	Check vents at rear of unit. Make sure that they are not blocked or covered.
CHANGE BULB	Bulb reached 200 hour lifetime limit	Yes Hold Alarm Reset button for 5 - 10 seconds. Reset to clear alarm	Change bulb.

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